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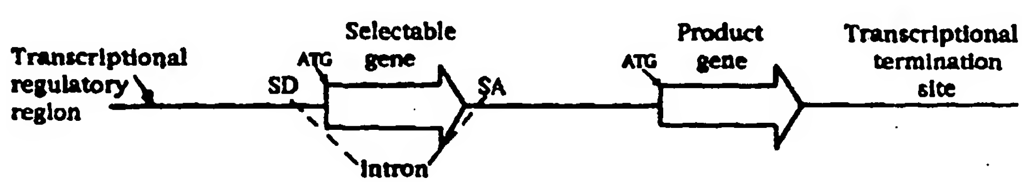

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., **264(24)**: 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (ΔGT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [ΔGT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ΔGT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	$Na^{+}K^{+}$-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5′ end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3′ end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerase chain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Req., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton *et al.*, Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen *et al.*, Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen *et al.*, Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen *et al.*, Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica *et al.*, Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter *et al.*, Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), *i.e.* Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR^{+} cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR' cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT 1000

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC 2300

ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC 2350

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT 3400

TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

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GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG 6800

CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT 6900

CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

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AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC 1600

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT 3150

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC 3300

GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT 4350

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

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TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA 5950

AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 2050

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 2100

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT 2150

TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA 2200

GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG 2250

GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC 2300

ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2350

GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC 2400

GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT 2450

CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC 2500

GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG 2550

CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG 2600

AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT 2650

ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA 2700

ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC 2750

CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC 2800

TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT 2850

TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA 2900

GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT 2950

GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG 3000

TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC 3050

GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA 3100

CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT 3150

TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC 3200

CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC 3250

CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG 3300

CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA 3350

CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA 3400

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CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG 3550

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CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG 3750

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AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA 4300

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GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG 4650

TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC 4700

ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA 4750

TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG 4800

AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC 4850

AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA 4900

CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT 4950

GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC 5000

TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC 5050

GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT 5100

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CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC 5200

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AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5900

GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5950

TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6000

CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6050

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AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT 6150

ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG 6200

GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT 6250

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CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG 6650

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG 6700

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6750

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6800

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

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TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA 7000

GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG 7050

TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG 7100

CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA 7150

AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG 7200

CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

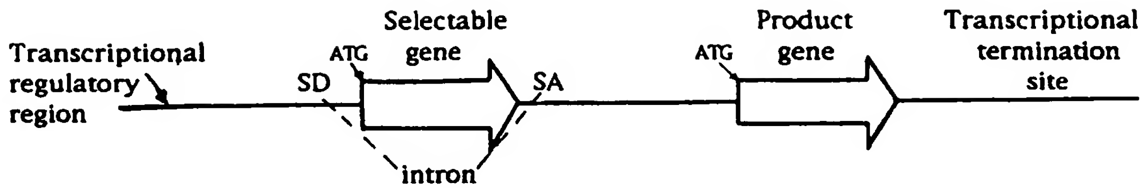

FIG. 1B

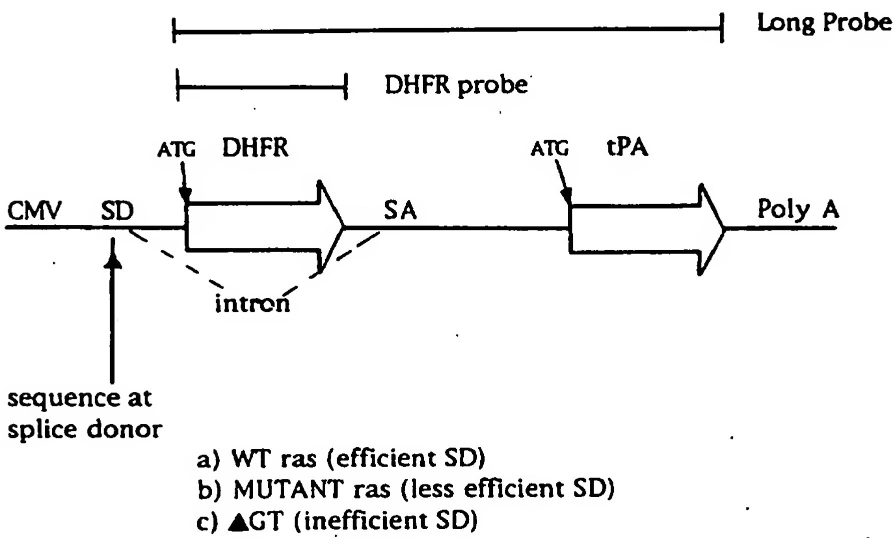

FIG. 1C

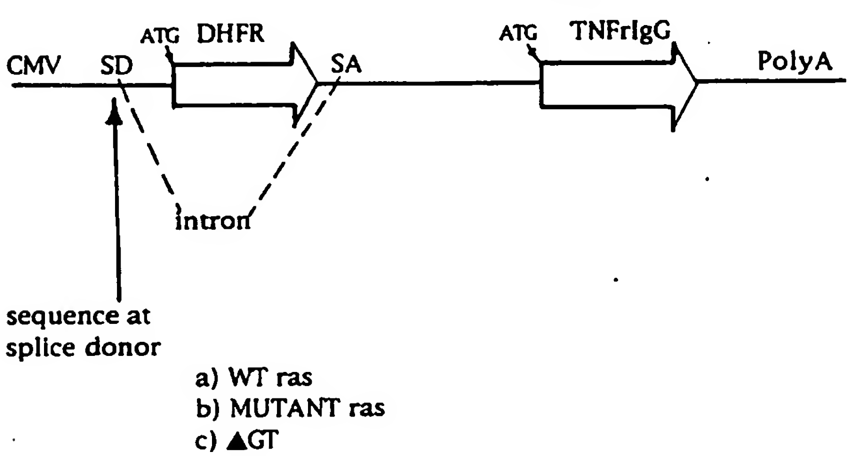

FIG. 1D

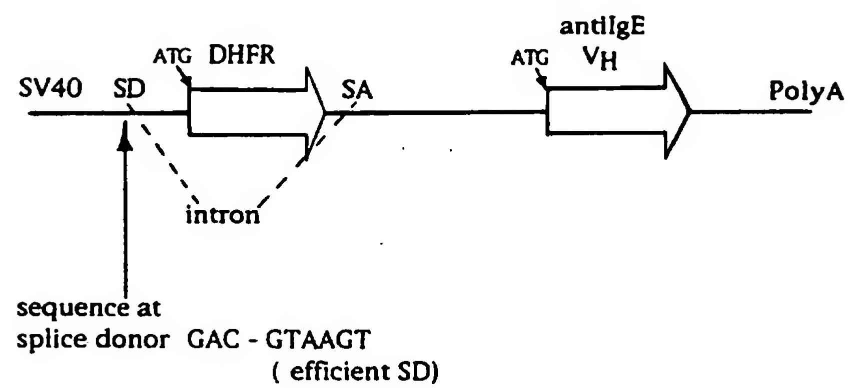
antiIgE
ATG DHFR
ATG V_H
SV40 SD
SA
PolyA
intron
sequence at
splice donor GAC - GTAAGT
(efficient SD)

FIG. 2

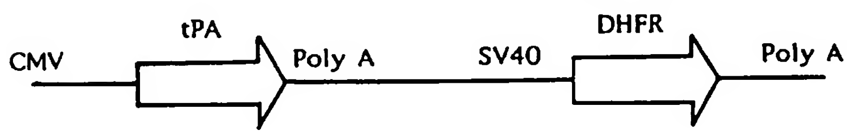
tPA
DHFR
CMV
Poly A
SV40
Poly A

FIG. 3A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI    aseI/asnI/vspI                       bslI             aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                  maeII
                sau96I                                     hinlI/acyI
                haeIII/palI     aciI                       ahaII/bsaHI
                asuI  apyI[dcm+]               aciI        aatII         maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                     maeII
      hinlI/acyI                                                                                hinlI/acyI
      ahaII/bsaHI                                    rsaI                        rsaI           ahaII/bsaHI
      aatII                              bglI        csp6I           ndeI        csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                     nlaIII
         bglI  dsaV                                                                          styI
        sau96I bstNI            rsaI                                         maeII                 ncoI
        haeIII/palI             csp6I                                   rsaI snaBI                 dsaI hphI  aciI
        asuI   apyI[dcm+]    bsrI nlaIII                                csp6I bsaAI                bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 3B

```
                                                                            maeII
                                                                           hinlI/acyI                 nlaIV
           rsaI                       pleI                                 ahaII/bsaHI                hgiCI
           csp6I                aciI  hinfI              bsmAI             aatII                      banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                      aluI
                                                                                                     sstI
                                                                                                     sacI
                                                                                                     hgiJII
                                                                                                     hgiAI/aspHI
                                                                                                     ecl136II
                                                                                                     bsp1286
                                                                                                     bsiHKAI
                                                                       rsaI                          bmyI
                          maeIII    aciI      hgaI       aciI          csp6I     mnlI                banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                       haeIII/palI
                                                                                      mcrI
                                                                                      eagI/xmaIII/eclXI
                                                                                      eaeI
                                                                                      cfrI
                                                                                     fnu4HI
                                                                                     aciI
                                                                                    thaI
                          esp3I                                                     fnuDII/mvnI
                     scrFI                                     sau96I          sacII/sstII
                     mvaI bsmAI                                     avaII           nspBII
                     ecoRII                                    asuI            kspI scrFI
                     dsaV                                     nlaIV            dsaI nciI
                     bstNI  hinlI/acyI                            scrFI         bglI bslI mspI
                     apyI(dcm+)                                   nciI     sau3AI mnlI bstUI
                sau3AI gsuI/bpmI                                  mspI     mboI/ndeII(dam-) hpaII
                mboI/ndeII(dam-)                             mboII  hpaII    dpnI(dam+) bsaJI dsaV
                dpnI(dam+)  hgaI  fokI                       bpuAI  dsaV      dpnII(dam-) bsh1236I
                dpnII(dam-) ahaII/bsaHI          mnlI        bbsI   cauII     alwI(dam-) aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 3C

```
                tfiI
            aciI
           thaI hinfI
           fnuDII/mvnI                aciI                                fnu4HI
           bstUI                   rsaI                                   bbvI
           bsh1236I                                                     nspBII           taqI
                                   csp6I  scfI           mnlI           aciI       nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                  thaI
                                                                                  fnuDII/mvnI
                                                                                  bstUI
                                                                                  bsh1236I
                                                                                mluI
                      pflMI                                         bsrBI       aflIII     rsaI
     sfaNI            bslI                             bsmAI        aciI      xmnI         csp6I
                                                       bsaI      mnlI   ddeI  asp700      scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                            scrFI
                                                            mvaI
          eco57I                                            ecoRII
         mboII                 tfiI                         dsaV
        earI/ksp632I           hinfI                        bstNI                  tfiI       tru9I         tru9I
       mnlI                  alwNI    hphI                  apyI[dcm+]           mboII  taqI  mseI          mseI
                                                          sexAI            ddeI    hinfI     ahaIII/draI   aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                    tru9I
                                             banII                                                    mspI
                                                                            sfaNI    mseI                 hpaII
          ddeI                 bslI    mnlI                 bstXI      fokI         aflII/bfrI        bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 3D

```
                                                                               haeIII/palI
                                                                              haeI
                                                       scrFI          scrFI                        nlaIII
                                                       mvaI                 mvaI                            sau3AI
                                                       ecoRII         ecoRII               mboI/ndeII[dam-]
                                                       dsaV   tfiI    dsaV    pleI              dpnI[dam+]
                                                       bstNI     nlaIII    bstNI     ddeI                 dpnII[dam-]
             accI  nlaIII             mnlI             apyI[dcm+]   hinfI  apyI[dcm+]    hinfI    maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI        scrFI
                                                                                     ahaII/bsaHI       mvaI
                                                                                  scrFI           ecoRII
                                                                                  mvaI    ecoNI        dsaV
                                                                                  ecoRII      sau96I
                                                                                  dsaV        avaII
                         maeII                                                    bstNI   bslI      asuI    mnlI
                       aflIII                                                     apyI[dcm+]     mnlI  bstNI
         apoI       maeIII                                     mnlI           bsaJI  hgaI      ddeI    apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                   ppu10I
                                                             sfaNI                                 nsiI/avaIII
         sfaNI                  accI     mboII            mboII                          mnlI     aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                         fnu4HI
        nlaIII                                           aciI
       styI                                             thaI
       ncoI                                             fnuDII/mvnI tru9I
       dsaI                           styI              bstUI       mseI                                     maeIII
       bsaJI                          bsaJI             bsh1236I   aseI/asnI/vspI                            hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

```
                                                        scrFI
                                                        mvaI
                                                        ecoRII                           bspMI
                                                        dsaV                           scfI
                                                        bstNI                               pstI
                                                        apyI[dcm+]      mnlI        aluI     bsgI               tru9I
                                                      gsuI/bpmI         bsaJI      hindIII fnu4HI               mseI
       scfI        fokI                           maeIII     hincII/hindII      ddeI       bbvI             apoI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATTCGAACCC GACGTCCAGC GGCACTTAAA

                                               hgiJII                                                 scrFI
                                               bsp1286                                                mvaI
                                               bmyI               fnu4HI                              ecoRII
                               sfaNI         mnlI                 bbvI               mboII            dsaV
                              fokI      mboII  banII          fnu4HI               bpuAI             bstNI
         hgaI              nlaIII      earI/ksp632I           bbvI                 bbsI              apyI[dcm+] nlaIII
1601 AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC
     TTCCCTGCGA CACTTCGTTA GTACCTACGT TACTTCTCTC CCGAGACGAC ACACGACGAC GACACACCTC GTCAGAAGCA AAGCGGGTCG GTCCTTTAGG

                                                 scfI
                               sau3AI            pstI
                               mboI/ndeII[dam-]  bsgI                                                          hgiAI/aspHI
                       nlaIV   dpnI[dam+]    sau3AI                                                    hinPI  bsp1286
                     mnlI     bstYI/xhoII    mboI/ndeII[dam-]                                      fnu4HI     bsiHKAI
           tfiI   mboII       bglII          dpnI[dam+]                                            bbvI       bmyI
           hinfI  earI/ksp632I dpnII[dam-]   dpnII[dam-]                                       nlaIII  hhaI/cfoI
1701 ATGCCCGATT CAGAAGAGGA GCCAGATCTT ACCAAGTGAT CTGCAGAGAT GAAAAAACGC AGATGATATA CCAGCAACAT CAGTCATGGC TGCGCCCTGT
     TACGGGCTAA GTCTTCTCCT CGGTCTAGAA TGGTTCACTA GACGTCTCTA CTTTTTTGCG TCTACTATAT GGTCGTTGTA GTCAGTACCG ACGCGGGACA

                  scrFI
                  nciI
                  mspI
                  hpaII                                              alwNI
                  dsaV                               bsp1286       ddeI                  fnu4HI   styI
      ddeI        cauII    sspI                      bmyI        draIII                  bbvI     bsaJI
1801 GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC
     CGAGTCTTCG TTGGCCCACC TTATAACGAC CACGTTGTCA CCGTCCCGTG TCACGGTGAG TCACGGACAG TTTTCAACGT CGCTCGGTTC CACAAAGTTG
```

FIG. 3F

```
                                                                                                        pmlI
                                                                                                          eco72I
                                                                                                      sau96I
                                                                                                      asuI maeII
                                                                                                  scrFI
                                                                                                  mvaI haeIII/palI
                                                                                                  ecoRII
                                                                                                  dsaV
       bspMI                                                                                      bstNI    bsaAI
    nlaIV           sau96I                                                                        bsaJI    bbrPI
    hgiCI           haeIII/palI                                                                   apyI[dcm+]
    banI            asuI   rsaI                          bsp1286
   bsp1286         eco0109I/draII                        bmyI
   bmyI           alwNI    csp6I ddeI                 bsrI       bcgI
1901 GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT
     CCCCCGTGGA CGGTCGTCCG GGACATGAAG AGTCTAAAGC ACACGGTCAC GGGGCTTCCT AAACGACCCT TCACGACACT TTATCTATGG TCCCGGTGCA

                scrFI                                     hinPI
                mvaI                                            hhaI/cfoI
                ecoRII                                   nlaIV    hgiAI/aspHI
                dsaV                                     narI     bsp1286                   thaI
                bstNI                  pmlI  hgiAI/aspHI       kasI     bsiHKAI                  fnuDII/mvnI
                bsaJI                  eco72I                  hinlI/acyI                        bstUI sau96I
            sau96I                bsaAI bsp1286                hgiCI    bmyI                 hinPI haeIII/palI
            avaII                 bbrPI bsiHKAI                haeII    apaLI/snoI           hhaI/cfoI
            asuI     sfaNI   scfI  bsp1286    bmyI     aciI        banI     alw44I/snoI        fnu4HI     asuI      bslI
          mnlI  apyI[dcm+] aluI     bmyI maeII      nspBII         ahaII/bsaHI      bsrI       bbvI bsh1236I      bslI
2001 GCTACGAGGA CCAGGGCATC AGCTACAGGG GCACGTGGAG CACAGCGGAG AGTGGCGCCG AGTGCACCAA CTGGAACAGC AGCGCGTTGG CCCAGAAGCC
     CGATGCTCCT GGTCCCGTAG TCGATGTCCC CGTGCACCTC GTGTCGCCTC TCACCGCGGC TCACGTGGTT GACCTTGTCG TCGCGCAACC GGGTCTTCGG

                                            scrFI
                                            mvaI
                                            ecoRII
                                            dsaV                                        pleI       scrFI
                                            bstNI                                          bsmAI        mvaI
                                            bsaJI                                         taqI[dam-]    ecoRII
                           hinlI/acyI       apyI[dcm+]                                  sau3AI hinfI    dsaV
                aciI  haeIII/palI        haeIII/palI              scfI                  mboI/ndeII[dam-] bstNI
      nspBII      haeI  hgaI bstXI      sau96I          pstI                     dpnI[dam+]        apyI[dcm+]      tru9I
     scfI aciI    mnlI   ahaII/bsaHI    asuI       nlaIV          bsgI           dpnII[dam-]          bsaJI       maeII mseI
2101 CTACAGCGGG CGGAGGCCAG ACGCCATCAG GCTGGGCCTG GGGAACCACA ACTACTGCAG AAACCCAGAT CGAGACTCAA AGCCCTGGTG CTACGTCTTT
     GATGTCGCCC GCCTCCGGTC TGCGGTAGTC CGACCCGGAC CCCTTGGTGT TGATGACGTC TTTGGGTCTA GCTCTGAGTT TCGGGACCAC GATGCAGAAA
```

FIG. 3G

```
                                   fnu4HI
                                   bbvI
                     aluI          scfI
                 rsaI            pstI                    mnlI       maeIII                          dsaI
        aciI     csp6I ddeI      bsgI                  ddeI       alwNI                             bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
     TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
                                                                                                    scrFI
                                                                                                    pflMI
                                                                                                    mvaI
                                                                                                    ecoRII
                                                                                                    dsaV
                                                       sau3AI                                       bstNI
                                                       mboI/ndeII[dam-]                             bslI
                                                       dpnI[dam+]                                   apyI[dcm+] haeIII/palI
                 pleI  nlaIV                 ecoRI     dpnII[dam-]                              bsp1286       sau96I
           hphI  hinfI hgiCI            dsaI apoI      alwI[dam-]                               bmyI alwNI    asuI
         mnlI   bcgI   banI mnlI   mnlI bsaJI      nlaIII                                    bsrI  bsaJI  bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
     TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
                                                            nlaIV
                                                            hgiCI
                                                            banI  maeII
                                                        scrFI
     scrFI                                        mvaI     pmlI
     mvaI                                               ecoRII
     ecoRII                                       dsaV
     dsaV                tfiI                     bstNI    eco72I
     bstNI                     hinfI              pflMI    bsaAI                               rsaI
     bsaJI                  mspI             sfaNI      apyI[dcm+]        mboII                      csp6I      bsp1286
     apyI[dcm+]             hpaII           fokI  bslI bsaJI     bbrPI  eco57I  aciI       maeII   scaI       bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
     GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
                                                                                                    scrFI
                                                                           mboII                    mvaI
                                                                          earI/ksp632I              ecoRII
                                                                       hgiJII                       dsaV
                                                                       bsp1286                      bstNI
                      fnu4HI  bsmAI                                    bmyI                         apyI[dcm+]
                      aciI  ddeI    rsaI         ddeI                  banII                  bslI bsaJI     fnu4HI
      mnlI        bspMI haeIII/palI csp6I      mnlI       sfaNI     mnlI sapI            mnlI    bslI        bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
     GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC
```

FIG. 3H

```
                                  mspI
                                  hpaII                                                                   scrFI
                                  bslI                                                                    mvaI
                                 scrFI                                                                    ecoRII
                                 nciI       nlaIV                                                         dsaV
                                 dsaV     aciI                       aluI        tfiI        aciI         bstNI
              bslI       mnlI    cauII  bsrBI    bslI aciI              alwNI        hinfI      fnu4HI        apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                    scrFI
                                                                    mvaI
                                                                    ecoRII
                                                                    dsaV
                                                                    bstNI
                                                                    apyI[dcm+]
                                                         scrFI     bslI
                                      sau3AI             nciI      bsaJI
                                      mboI/ndeII[dam-]   mspI   sau96I
                                      dpnI[dam+]         hpaII  nlaIV
                                      dpnII[dam-]        dsaV   avaII         mnlI
        mnlI    aciI    bslI      hphI[dam-]             cauII  asuI       mnlI         apoI       taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                         scfI                            scrFI
                                                         pstI                            mvaI
                                                        fnu4HI                           ecoRII
                                                        bbvI pvuII                       dsaV
                                                     fnu4HI fnu4HI                       bstNI          aciI
                                                     bbvI   bbvI                    bsp1286      fnu4HI  sau96I
              ecoRI                                hinPI bsgI aluI        tfiI     nspBII  bsaJI    bbvI    avaII
              apoI taqI                            hhaI/cfoI nspBII       hinfI    aciI bmyI apyI[dcm+]     asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                                aluI
                                                               sstI
                                    pvuII                      sacI
                       mspI      scfI                          hgiJII
                       hpaII     pstI  fnu4HI                  hgiAI/aspHI
                      scrFI    bspMI   bbvI                    ecl136II                    haeIII/palI
                      nciI   sau96I nspBII                     bsp1286                    stuI
                      dsaV   avaII fnu4HI      bsrI            bsiHKAI                    haeI
                      cauII  asuI  bbvI    mspI                bmyI    mspI              mnlI
      draIII    bslI bsaJI aciI  bsgI aluI hpaII               banII   hpaII          nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                     fnu4HI
                                                   nspBII
        fnu4HI                                     aciI                                             nlaIII
        aciI                          rsaI fokI   fnu4HI                    tru9I        hphI      nspI
      bsrBI eco57I mnlI  nlaIII       csp6I    alwNI bbvI                   mseI        maeIII     nspHI        gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                         sau96I
                         nlaIV
                         haeIII/palI
                         asuI
                        sau96I
                        nlaIV scrFI                                      scrFI
                        hgiJII                                                 mvaI
                        eco0109I/draII                  scrFI            ecoRII
                        bsp1286                         mvaI                   dsaV
                        bsp120I                         ecoRII           bstNI
                        bmyI  mvaI                      dsaV        sau96I
                        banII ecoRII              bstNI             nlaIV                       aciI
                      aciI    dsaV                bsaJI             haeIII/palI                fnu4HI
                   fnu4HI     bstNI            hinlI/acyI       tfiI      asuI apyI[dcm+]           haeIII/palI
                   aciI asuI  apyI[dcm+]       hgaI     apyI[dcm+]       eco0109I/draII            eaeI
      bsmAI     bsrBI  apaI bsaJI              ahaII/bsaHI      hinfI   mnlI  bsaJI                cfrI   nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        eco0109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII                tfiI       nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI            rmaI     hinfI       nspHI
           sfaNI   nspBII  haeIII/palI           fokI  avaI     bsp1407I  bstEII        maeI bsrI      maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                             mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                       mboI/ndeII[dam-]
                dsaV                         dpnI[dam+]            mboII
                bstNI           mnlI               dpnII[dam-]     eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]     mboII  bpuAI           hinPI
      mcrI maeIII            hinfI                bstYI/xhoII  mboII    bbsI            hhaI/cfoI      scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI               mboII
      gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI earI/ksp632I                                    tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                   scrFI
                                                                 scrFI       mvaI
                                                                 mvaI bsmI         ecoRII
                                                                 ecoRII      dsaV
                                                                 dsaV        bstNI
                                      mboII                      bstNI             bsaJI
                                      bpuAI   bsmI      rmaI   gsuI/bpmI   mnlI   bsaJI                ecoRI  taqI
                                      bbsI nlaIII       maeI mnlI apyI[dcm+]  mnlI apyI[dcm+]          apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
        styI
      aciI
     fnu4HI   sau96I
     bglI nlaIII
    sfiI ncoI haeIII/palI
    haeIII/palI                    aluI
   eaeI  dsaI asuI              fnu4HI
   cfrI  bsaJI                  bbvI          maeIII                sfaNI  apoI                          bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                        sau3AI
                                                        mboI/ndeII[dam-]
                                                        dpnI[dam+]
                                                        dpnII[dam-]
                                                       pvuI/bspCI
                                                       mcrI
                                                      taqI[dam-]  tru9I
                                                    claI/bsp106[dam-]          styI
                                                   sau3AI       mseI     fnu4HI    haeI
                                                   mboI/ndeII[dam-]      bbvI   ncoI
                                                   dpnI[dam+] xmnI     hinPI    dsaI haeIII/palI
    rmaI                                           dpnII[dam-]  aseI/asnI/vspI  bsaJI
    maeI                                    nlaIII alwI[dam-]  asp700   hhaI/cfoI nlaIII      mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                 rsaI                                                                     nlaIV
                 csp6I                                                                 scrFI
                nlaIV                                                                  mvaI
                kpnI                                                                   ecoRII
                hgiCI                                                                  dsaV
                banI                   aluI                                            bstNI
                asp718     mnlI       pvuII                                            apyI[dcm+]
    mnlI        acc65I   ddeI aciI    nspBII                                          bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                                          nlaIV
           sfaNI                         scrFI          scrFI                                    sfaNI
          ppu10I                         mvaI                 mvaI                                         ppu10I
          nsiI/avaIII                    ecoRII         ecoRII                                 nsiI/avaIII
         nlaIII                          dsaV           dsaV                                  nlaIII
        sphI                             bstNI                bstNI                                     sphI
        nspI                             apyI[dcm+]           apyI[dcm+]                                nspI
        nspHI                           sexAI                bsaJI                                      nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                             nlaIII
                                                                                            styI
                                                                                            ncoI
                                          aciI                                        bslI  dsaI
           aciI          aciI  fokI                    aciI bsrI   aciI               aciI  bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                                   rmaI
                                                                                  styI
           aciI                                                                   bsaJI
          fnu4HI                                                                  blnI
         sfiI                                                                     avrII
         haeIII/palI                                                            haeIII/palI           tru9I
     bsaJI bglI          ddeI                                                  stuI                  hpaI
   haeIII/palI mnlI haeIII/palI                             mnlI               haeI.                 hincII/hindII
  mnlI    mnlI   bsaJI   mnlI   aluI                     mnlI                 mnlI maeI          aluI mseI   aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                           scrFI
                                           mvaI
                                           ecoRII
   haeIII/palI                             dsaV
  eaeI                                     bstNI                                                     aluI
  cfrI                        bsrI         apyI[dcm+]       tru9I       fnu4HI                      pvuII
 bsrI                  maeII maeIII       bsaJI    maeIII   mseI        bbvI    fokI                nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

```
                                                                   hinPI
                                                                   hhaI/cfoI
                                                                  nlaIV
              sau3AI                                              narI
     sau96I   mboI/ndeII[dam-]                                    kasI
     haeIII/palI                                                  hinlI/acyI
     asuI     dpnI[dam+]                                          hgiCI
   mnlI       dpnII[dam-]                                         haeII    aciI
 mboII  aciI  pvuI/bspCI                                          banI   sfaNI
 earI/ksp632I mcrI                        bglI                    ahaII/bsaHI                 sfaNI    aciI
4301 CGAAGAGGCC CGCACCGATC GCCCTTCCCA ACAGTTGCGT AGCCTGAATG GCGAATGGCG CCTGATGCGG TATTTTCTCC TTACGCATCT GTGCGGTATT
     GCTTCTCCGG GCGTGGCTAG CGGGAAGGGT TGTCAACGCA TCGGACTTAC CGCTTACCGC GGACTACGCC ATAAAAGAGG AATGCGTAGA CACGCCATAA

                                                                     aciI
                                                                   fnu4HI                fnu4HI
                                  hinPI                           thaI                 hinPI
                                 thaI                             fnuDII/mvnI          hhaI/cfoI
                                 fnuDII/mvnI                      bstUI                thaI
                                 bstUI scfI     hinPI            hinPI                 fnuDII/mvnI
                                 bsh1236I       hhaI/cfoI hhaI/cfoI                    bstUI
                              rsaI hhaI/cfoI  fnu4HI    tru9I aciI                     bsh1236I     aciI
    aciI    maeII             csp6I   bslI    aciI      mseI bsh1236I               maeIII bbvI  maeIII
4401 TCACACCGCA TACGTCAAAG CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT GGTTACGCGC AGCGTGACCG CTACACTTGC
     AGTGTGGCGT ATGCAGTTTC GTTGGTATCA TGCGCGGGAC ATCGCCGCGT AATTCGCGCC GCCCACACCA CCAATGCGCG TCGCACTGGC GATGTGAACG

           hinPI
           hhaI/cfoI                                                                          nlaIV
       rmaI                                                        mspI                       hgiJII
  hinPI  haeII                                                     hpaII                      bsp1286
  hhaI/cfoI   bsrBI                                               naeI                        bmyI
 haeII maeI   aciI            mboII                    maeII  cfr10I           aluI           banII         nlaIV
4501 CAGCGCCCTA GCGCCCGCTC CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCGG CTTTCCCCGT CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG
     GTCGCGGGAT CGCGGGCGAG GAAAGCGAAA GAAGGGAAGG AAAGAGCGGT GCAAGCGGCC GAAAGGGGCA GTTCGAGATT TAGCCCCCGA GGGAAATCCC

                nlaIV                                               maeII    haeIII/palI
                hgiCI  taqI                                        draIII   sau96I
                banI mnlI                             hphI         bsaAI    asuI
4601 TTCCGATTTA GTGCTTTACG GCACCTCGAC CCCAAAAAAC TTGATTTGGG TGATGGTTCA CGTAGTGGGC CATCGCCCTG ATAGACGGTT TTTCGCCCTT
     AAGGCTAAAT CACGAAATGC CGTGGAGCTG GGGTTTTTTG AACTAAACCC ACTACCAAGT GCATCACCCG GTAGCGGGAC TATCTGCCAA AAAGCGGGAA

  maeII pleI        tru9I     pleI                                  bslI
 drdI   hinfI maeII mseI      hinfI        bsrI                    bslI  avaI
4701 TGACGTTGGA GTCCACGTTC TTTAATAGTG GACTCTTGTT CCAAACTGGA ACAACACTCA ACCCTATCTC GGGCTATTCT TTTGATTTAT AAGGGATTTT
     ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACAA GGTTTGACCT TGTTGTGAGT TGGGATAGAG CCCGATAAGA AAACTAAATA TTCCCTAAAA
```

FIG. 3N

```
                                                               thaI                                                  hgiAI/aspHI
                                                               fnuDII/mvnI              maeII                        bsp1286
                                                               tru9I   apoI tru9I        psp1406I                     bsiHKAI
              tru9I                                            mseI bstUI  mseI        tru9I                         bmyI   ddeI
              mseI                          tru9I                                                                    apaLI/snoI
         haeIII/palI              aluI      mseI      apoI     bsh1236I         sspI mseI                            alw44I/snoI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
     CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG

                                                                                                                        hinPI
                                                                                                                        hhaI/cfoI
                                                                                                                       thaI
                                                                               hinPI                                   fnuDII/mvnI
              aciI                                           maeIII          fnu4HI                                    bstUI
 rsaI        fnu4HI     tru9I                             maeII bsrI      nlaIII hhaI/cfoI                       nspBII bsh1236I
 csp6I      sfaNI       mseI        aciI                 bsaAI tth111I/aspI bbvI                        aciI     aciI hgaI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
     TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG

                                                                     scrFI
                       sfaNI                                         nciI
                    mspI                                             mspI
                    hpaII                                            hpaII    nspI
                   scrFI                                             dsaV     nspHI
                   nciI                                    esp3I          fnu4HI
                   dsaV fokI                          maeIII bsmAI        bbvI
       drdI        cauII   aciI               aluI     bslI  cauII aluI nlaIII    mnlI    hphI     hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
     CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT

  thaI
  fnuDII/mvnI
  bstUI
  bsh1236I
 hinPI
 hhaI/cfoI                         mnlI
thaI mnlI                        haeIII/palI                                                          hinlI/acyI
fnuDII/mvnI          mboII      sau96I                                      nlaIII                    ahaII/bsaHI
bstUI                bpuAI      asuI                                 tru9I  rcaI                      aatII
bsh1236I             bbsI      eco0109I/draII                        mseI   bspHI                 ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
     GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT
```

FIG. 3O

```
                            nlaIV
                         aciI
                        thaI
                        fnuDII/mvnI
                        bstUI                                                    rcaI
                        bsh1236I                                                 bspHI
                       hinPI                                                 bsrBI     bsmAI
                       hhaI/cfoI                                             aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                      mboII                                                 fnu4HI
        sspI          earI/ksp632I                                          aciI                          hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                              hgiAI/aspHI
                                              bsp1286
                                   sau3AI     bsiHKAI                      sau3AI   nspBII    sau3AI
                                   mboI/ndeII[dam-]                        mboI/ndeII[dam-]   mboI/ndeII[dam-]
                                   dpnI[dam+] bmyI                         dpnI[dam+]         dpnI[dam+]
                                   dpnII[dam-]                            bstYI/xhoII         dpnII[dam-]
                              eco57I          apaLI/snoI              bsrI dpnII[dam-]        alwI[dam-]
           hphI          sfaNI  mboII[dam-]   alw44I/snoI  maeIII  taqI   alwI[dam-]   aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                                     scrFI
                                                             aciI                    nciI
                                                           thaI                      mspI
             maeII                                         fnuDII/mvnI               hpaII
            psp1406I          hgiAI/aspHI                  bstUI                     dsaV
           xmnI               bsp1286   tru9I              bsh1236I             hinlI/acyI
           asp700             bsiHKAI   mseI              hinPI                 hgaI cauII                    aciI
        mboII                 bmyI   ahaIII/draI          hhaI/cfoI             ahaII/bsaHI     bcgI  mcrI   fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                                   rsaI
                                   csp6I   bsrI                                                       fnu4HI
              ddeI                scaI  hphI  maeIII      sfaNI     fokI   nlaIII                     bbvI        nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

```
                                                      sau96I
                                                      avaII
                                              sau3AI  asuI                                          nlaIII
                       haeIII/palI            mboI/ndeII[dam-]                                   sau3AI maeIII
                      eaeI                    dpnI[dam+]                                         mboI/ndeII[dam-]
                      cfrI                    dpnII[dam-]                                        dpnI[dam+]
                     fnu4HI                  pvuI/bspCI                                          dpnII[dam-]
                     aciI                    mcrI   mnlI         aluI   aciI             nlaIII alwI[dam-]
5701 ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC
     TACTCACTAT TGTGACGCCG GTTGAATGAA GACTGTTGCT AGCCTCCTGG CTTCCTCGAT TGGCGAAAAA ACGTGTTGTA CCCCCTAGTA CATTGAGCGG

                                                                                                  hinPI
                    mspI                                                                          hhaI/cfoI
       sau3AI   nlaIV                                                                            mstI
       mboI/ndeII[dam-] aluI                                                                     aviII/fspI    bsrI
       dpnI[dam+]   hpaII                                                   fnu4HI          maeII           tru9I
       dpnII[dam-] bsaWI                                 maeIII     sfaNI   bbvI           psp1406I         mseI
5801 TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC
     AACTAGCAAC CCTTGGCCTC GACTTACTTC GGTATGGTTT GCTGCTCGCA CTGTGGTGCT ACGGTCGTCG TTACCGTTGT TGCAACGCGT TTGATAATTG

                               mspI
                               hpaII                                                              bglI
                              scrFI                                                              sau96I
                        aluI  nciI        tru9I      fokI                     sau96I             haeIII/palI
                      rmaI    dsaV        mseI    bsrI        aciI            avaII       hinPI  asuI    mspI
                      maeI    cauII      aseI/asnI/vspI   mnlI                asuI        hhaI/cfoI      hpaII
5901 TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC
     ACCGCTTGAT GAATGAGATC GAAGGGCCGT TGTTAATTAT CTGACCTACC TCCGCCTATT TCAACGTCCT GGTGAAGACG CGAGCCGGGA AGGCCGACCG

                                mspI               thaI
                                hpaII              fnuDII/mvnI             haeIII/palI
                               cfr10I              bstUI                  sau96I
                             nlaIV hphI       bsmAI aciI        fnu4HI   nlaIV
                          gsuI/bpmI          bsaI  bsh1236I     bbvI bsrI asuI             mnlI
6001 TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT
     ACCAAATAAC GACTATTTAG ACCTCGGCCA CTCGCACCCA GAGCGCCATA GTAACGTCGT GACCCCGGTC TACCATTCGG GAGGGCATAG CATCAATAGA

                                                         ddeI
                                                    sau3AI        nlaIV
                 pleI                               mboI/ndeII[dam-]
                 hinfI                              dpnI[dam+]    hgiCI         tru9I
          eam1105I              fokI                dpnII[dam-]   banI mnlI     mseI       maeIII
6101 ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA
     TGTGCTGCCC CTCAGTCCGT TGATACCTAC TTGCTTTATC TGTCTAGCGA CTCTATCCAC GGAGTGACTA ATTCGTAACC ATTGACAGTC TGGTTCAAAT
```

FIG. 3Q

```
                                                             rmaI      sau3AI
                                                         sau3AI hphI   mboI/ndeII[dam-]
                                                         mboI/ndeII[dam-]
                                                         dpnI[dam+]    dpnI[dam+]
                                                         dpnII[dam-]   dpnII[dam-]
                             tru9I                tru9I bstYI/xhoII    alwI[dam-]        nlaIII              maeII
                             mseI         tru9I mseI  alwI[dam-]  bstYI/xhoII           rcaI             tru9I
                            ahaIII/draI     mseI ahaIII/draI maeI  mboII[dam-]          bspHI            mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                                       sau3AI
                                                       mboI/ndeII[dam-]
                                                       dpnI[dam+]  sau3AI
                                                       dpnII[dam-] mboI/ndeII[dam-] thaI
                                                      bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                              sau3AI  alwI[dam-]   dpnII[dam-]      bstUI
                                              mboI/ndeII[dam-]     alwI[dam-]       bsh1236I
                        hgaI                  dpnI[dam+] mboII[dam-]               hinPI       fnu4HI
                    ddeI                      dpnII[dam-]      bstYI/xhoII         hhaI/cfoI   bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                         sau3AI
                                         mboI/ndeII[dam-]
                                         dpnI[dam+]
                                         dpnII[dam-]
                                        alwI[dam-]
                    aciI                mspI                                   bsrI                 hinPI
          aciI     nspBII               hpaII        aluI                  maeIII    eco57I         hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                                          fnu4HI
                                                                                                alwNI     bbvI
                                                                                               bsrI  fnu4HI
         rmaI                haeIII/palI
         maeI     bslI      haeI                 scfI     aciI        mnlI                maeIII     bbvI      bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

```
                         scrFI                                       aciI                   hgiAI/aspHI
                         nciI                                      nspBII                   bsp1286
                         mspI                     mspI        fnu4HI                        bsiHKAI
                         hpaII                    hpaII       bbvI                          bmyI
                         dsaV     pleI           bsaWI      hinPI  mcrI                     apaLI/snoI
                         cauII    hinfI       maeIII        hhaI/cfoI                       alw44I/snoI   aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
     CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA

                                                                                                          mspI
                                                                 hinPI                                    hpaII
                                                                 hhaI/cfoI                                bslI   fnu4HI
                               ddeI      scfI                   haeII                        aciI        bsaWI  aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
     ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

                                              scrFI
                                              mvaI           scrFI
                                              ecoRII   mvaI
                                              dsaV     ecoRII
                                              bstNI          dsaV
                             hinPI mnlI       bsaJI          bstNI                                           taqI
                             hhaI/cfoI   aluI apyI[dcm+]     apyI[dcm+]                        mnlI drdI     hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
     GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA

                                                                                       haeIII/palI
                                                                   haeIII/palI      scrFI
                                                                 fnu4HI             mvaI bslI
                                                                 aciI               ecoRII
                                                                thaI bslI           dsaV                  nlaIII
                                                                fnuDII/mvnI         bstNI                     nspI
                                    nlaIV                       bstUI               apyI[dcm+]   haeIII/palI  nspHI
                 sfaNI            aciI                          bsh1236I       nlaIV haeI        haeI         aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
     GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT

                                                                                        fnu4HI              fnu4HI
                                                                                        bbvI                bbvI pleI
                               tfiI                                          bsrBI   aciI                 hinPI  hinfI
                               hinfI                   aciI          aluI    aciI   fnu4HI       mcrI     hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
     ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT
```

FIG. 3S

```
                                                                    thaI
                                                                     fnuDII/mvnI
                                                                     bstUI
                                                                     bsh1236I
                                                                    hinPI
                                                                    hhaI/cfoI
                                                                   thaI
                                                                   fnuDII/mvnI
                     sapI  hinPI                                   bstUI   haeIII/palI         aluI
                     mboII hhaI/cfoI                               bsh1236I            tru9I  pvuII
                     earI/ksp632I                  mnlI           bslI    eaeI   tfiI aseI/asnI/vspI
             mnlI  aciI    haeII                 aciI             aciI    cfrI   hinfI mseI    nspBII                bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
                                 tru9I                              nlaIV bstNI
                       hinPI     mseI      maeIII                   hgiCI apyI[dcm+]              mspI
          aciI         hhaI/cfoI aseI/asnI/vspI mnlI                banI bsaJI                    hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                                       tru9I
                                                                       mseI
                                                                      aseI/asnI/vspI
            aciI                                                    xmnI
          bsrBI                           aluI      nlaIII          asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT

>length: 7360
```

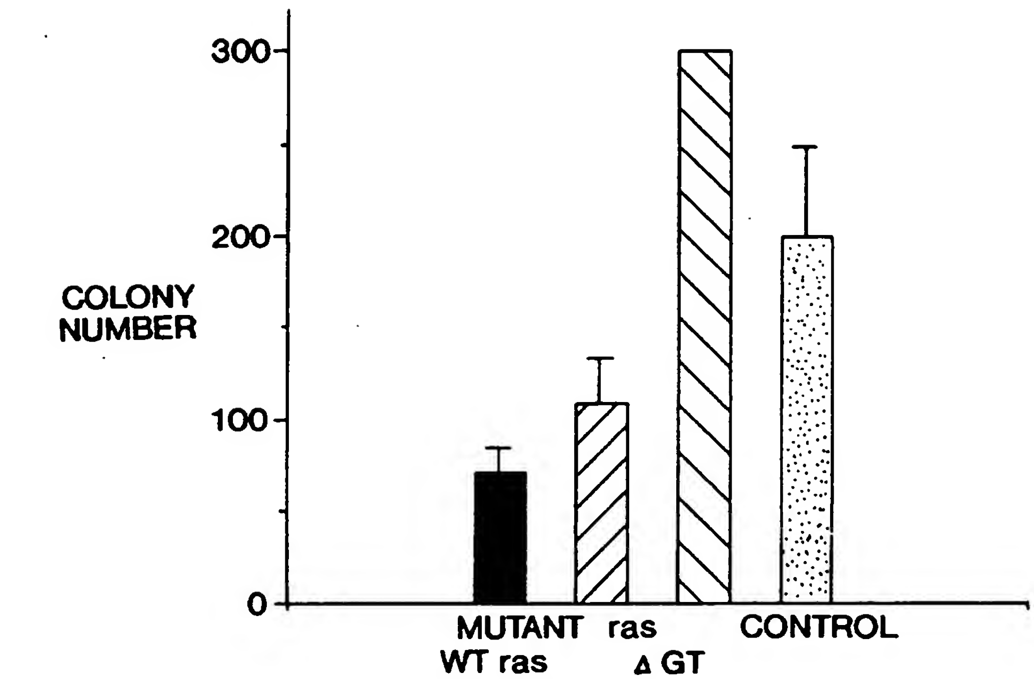
300
200
100
0
COLONY NUMBER
MUTANT
WT ras
ras
Δ GT
CONTROL

FIG. 4

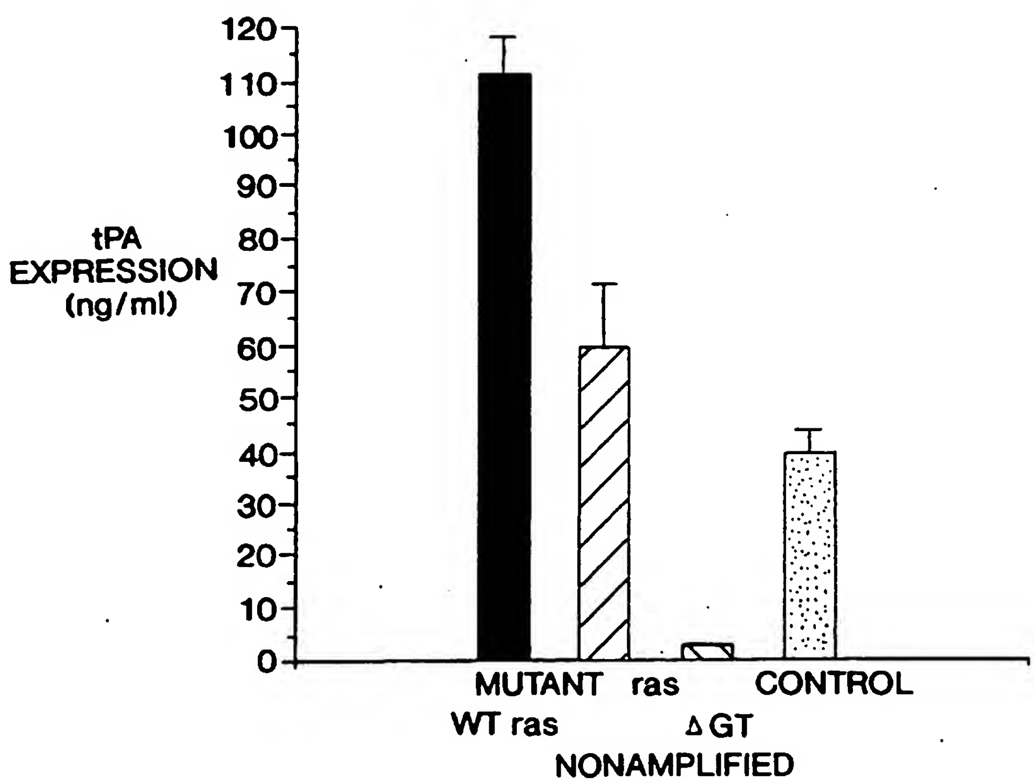
120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
MUTANT
WT ras
ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5A

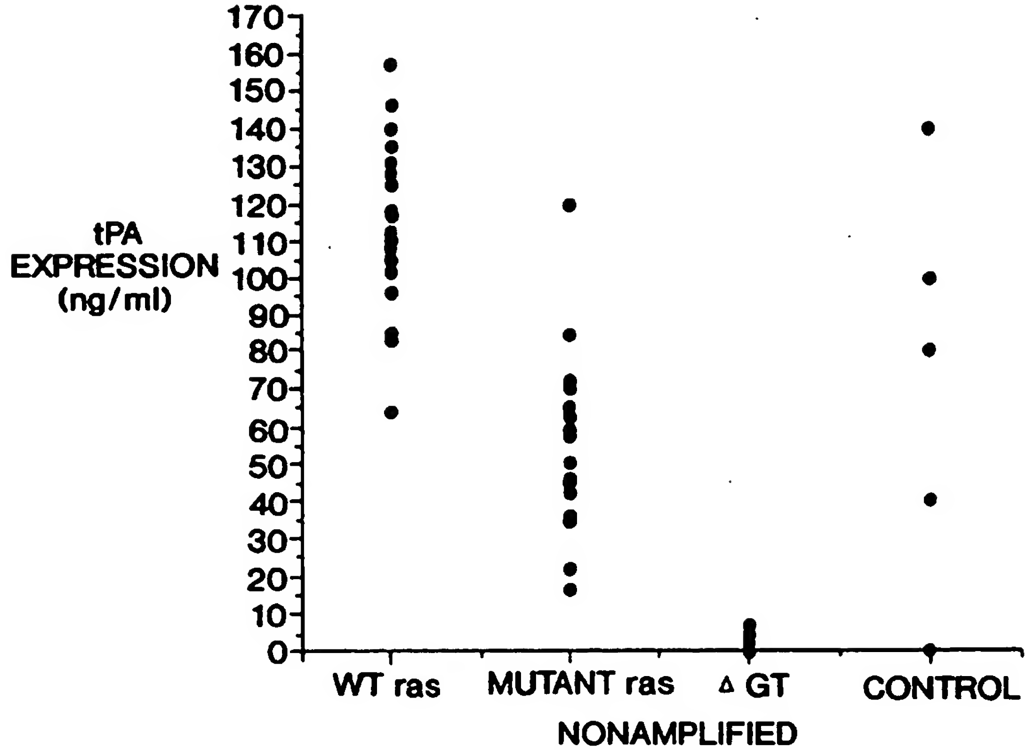
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
tPA EXPRESSION (ng/ml)
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

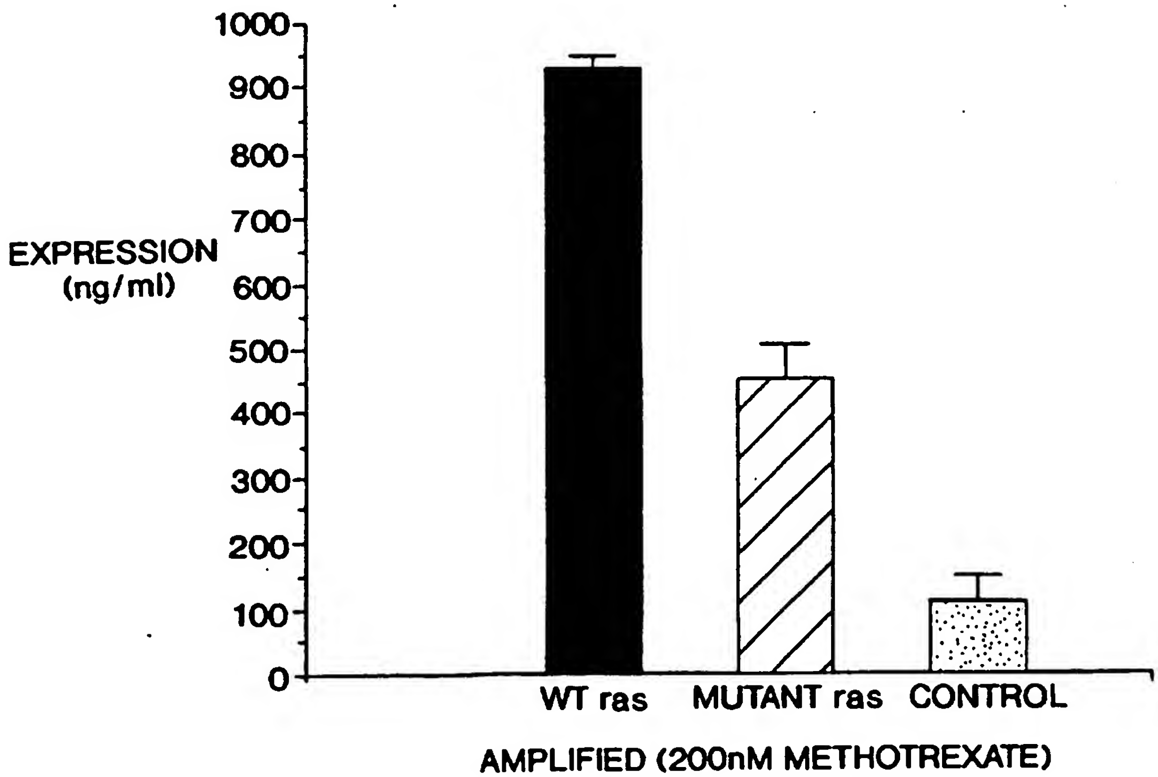
1000
900
800
700
600
500
400
300
200
100
0
EXPRESSION (ng/ml)
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 6A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnI
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI    aseI/asnI/vspI                           bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                  maeII
                sau96I                                     hinlI/acyI
                haeIII/palI     aciI                       ahaII/bsaHI
                asuI  apyI[dcm+]                aciI       aatII          maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                      maeII
      hinlI/acyI                                                                                 hinlI/acyI
      ahaII/bsaHI                                 rsaI                       rsaI                ahaII/bsaHI
      aatII                          bglI         csp6I           ndeI       csp6I               aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                     nlaIII
         bglI  dsaV                                                                              styI
        sau96I bstNI             rsaI                                       maeII                     ncoI
        haeIII/palI              csp6I                              rsaI   snaBI                      dsaI hphI  aciI
        asuI   apyI[dcm+]   bsrI  nlaIII                            csp6I  bsaAI                      bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 6B

```
                                                                                   maeII
                                                                                  hinlI/acyI              nlaIV
               rsaI                          pleI                                 ahaII/bsaHI             hgiCI
               csp6I                  aciI   hinfI                bsmAI           aatII                   banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                             rsaI                        bmyI
                                maeIII    aciI       hgaI        aciI        csp6I     mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                                haeIII/palI
                                                                                               mcrI
                                                                                               eagI/xmaIII/eclXI
                                                                                               eaeI
                                                                                               cfrI
                                                                                              fnu4HI
                                                                                              aciI
                                                                                             thaI
                              esp3I                                                          fnuDII/mvnI
                         scrFI                                       sau96I           sacII/sstII
                         mvaI bsmAI                                        avaII            nspBII
                         ecoRII                                      asuI             kspI  scrFI
                         dsaV                                       nlaIV             dsaI  nciI
                         bstNI  hinlI/acyI                              scrFI          bglI bslI  mspI
                         apyI[dcm+]                                     nciI      sau3AI mnlI bstUI
                    sau3AI gsuI/bpmI                                    mspI      mboI/ndeII[dam-] hpaII
                    mboI/ndeII[dam-]                             mboII  hpaII     dpnI[dam+] bsaJI dsaV
                    dpnI[dam+]  hgaI  fokI                       bpuAI  dsaV      dpnII[dam-] bsh1236I
                    dpnII[dam-] ahaII/bsaHI               mnlI   bbsI   cauII     alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 6C

```
                tfiI
            aciI
           thaI hinfI                                                        fnu4HI
           fnuDII/mvnI                 aciI                                  bbvI
           bstUI                    rsaI                                   nspBII           taqI
           bsh1236I                 csp6I   scfI           mnlI            aciI       nlaIII
  701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
      AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                 thaI
                                                                                 fnuDII/mvnI
                                                                                 bstUI
                                                                                 bsh1236I
                                                                               mluI
                                                                     bsrBI     aflIII      rsaI
                      pflMI                        bsmAI             aciI    xmnI          csp6I
      sfaNI           bslI                         bsaI           mnlI   ddeI asp700      scaI
  801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
      CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                           scrFI
                                                           mvaI
                                                           ecoRII
        eco57I                                             dsaV
       mboII                tfiI                           bstNI                   tfiI       tru9I         tru9I
      earI/ksp632I          hinfI                          apyI(dcm+)           mboII  taqI   mseI          mseI
     mnlI                 alwNI     hphI                 sexAI               ddeI    hinfI   ahaIII/draI   aseI/asnI/vspI
  901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
      TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                              aluI
                                             sstI
                                             sacI
                                             hgiJII
                                             hgiAI/aspHI
                                             ecl136II
                                             bsp1286
                                             bsiHKAI
                                             bmyI                                  tru9I               mspI
                                             banII                           sfaNI mseI                hpaII
           ddeI                   bslI    mnlI             bstXI      fokI       aflII/bfrI         bsaWI
 1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
      TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6D

```
                                                                                   haeIII/palI
                                                                                  haeI
                                                          scrFI         scrFI                          nlaIII
                                                          mvaI               mvaI                                 sau3AI
                                                          ecoRII        ecoRII                      mboI/ndeII[dam-]
                                                          dsaV   tfiI   dsaV   pleI                 dpnI[dam+]
                                                          bstNI    nlaIII    bstNI    ddeI                     dpnII[dam-]
           accI  nlaIII              mnlI                 apyI[dcm+]  hinfI  apyI[dcm+]   hinfI     maeIII alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                           mnlI
                                                                                       hinlI/acyI       scrFI
                                                                                       ahaII/bsaHI      mvaI
                                                                                    scrFI        ecoRII
                                                                                    mvaI   ecoNI        dsaV
                                                                                    ecoRII     sau96I
                                                                                    dsaV       avaII
                        maeII                                                       bstNI  bslI     asuI   mnlI
                      aflIII                                                        apyI[dcm+]    mnlI  bstNI
        apoI        maeIII                                      mnlI              bsaJI hgaI     ddeI   apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                                 ppu10I
                                                              sfaNI                              nsiI/avaIII
       sfaNI                    accI    mboII            mboII                       mnlI    aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                           fnu4HI
      nlaIII                                               aciI
     styI                                                 thaI
     ncoI                                                 fnuDII/mvnI tru9I
     dsaI                      styI                       bstUI      mseI                               maeIII
     bsaJI                     bsaJI                      bsh1236I  aseI/asnI/vspI                       hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6E

```
                                                                                                              haeIII/palI
                                                                                                            eaeI
                                                        scrFI                                        cfrI
                                                        mvaI                                              mspI
                                                        ecoRII                                     hpaII
                                                        dsaV                                      scrFI
                                                        bstNI                                             nciI
                                                        apyI[dcm+]       mnlI              ecoRI  dsaV
                                                      gsuI/bpmI          bsaJI       taqI  apoI   cauII
     scfI        fokI                              maeIII      hincII/hindII       claI/bsp106  bsaJI      aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                         gsuI/bpmI
                                                        scrFI
                                                        mvaI
                                                        ecoRII                                                  mnlI
                                                        dsaV                                                    ecoNI
         mnlI                                nspBII     bstNI                        ddeI                         sau96I
       haeIII/palI                       fnu4HI      hgiAI/aspHI                    mnlI                          nlaIV
      sau96I                          fnu4HI aciI    bsp1286                        eco81I                        avaII
      asuI                            bbvI  fnu4HI   bsiHKAI     aluI               bsu36I/mstII/sauI             asuI  hphI
   nlaIII                        bspMI   bbvI        bmyI apyI[dcm+]              bslI                         bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

   rmaI
   maeI
  styI
  bsaJI
  blnI            mboII                styI                                       rsaI
  avrII           earI/ksp632I         bsaJI            mnlI         taqI         csp6I              nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6F

```
                                  scrFI
                                  nciI
                                  mspI
                                  hpaII
                                  dsaV
                                  cauII
                                 xmaI/pspAI
                                 smaI
                                 scrFI
                                 nciI
                                 dsaV
                                 cauII
                                 bslI
                               sau96I
                               haeIII/palI
                               asuI
                            scrFI
                            mvaI bsaJI
                            ecoRII
                            dsaV                                        nlaIV
        rsaI .              bstNI bsaJI             scfI                    fnu4HI                               alwNI
        csp6I               bslI avaI               pstI                    aciI          aciI                ddeI
       bsp1407I             apyI[dcm+]              bsgI                 bsrBI       hphI   eco57I            mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                              scrFI
                                                         mboII                nciI
                                                        earI/ksp632I          mspI
       fnu4HI                                        sau3AI                   hpaII
      aluI                                           mboI/ndeII[dam-]         dsaV                 scfI               mspI
     pvuII                                           dpnI[dam+]               cauII                pstI               hpaII
     nspBII                                          dpnII[dam-]           sau96I                  bsgI            rsaI
   ddeI                                             bstYI/xhoII            avaII                  fnu4HI           csp6I
   mnlI bbvI                                        bglII                  asuI       draIII      bbvI    mboII   bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6G

```
                                                                          hgiAI/aspHI                          hgiAI/aspHI
                                                                          bsp1286      scrFI           bsp1286
                                                                          bsiHKAI      mvaI                   bsiHKAI
                                                     mnlI           sau96I             ecoRII          bmyI
                                                  alwNI             avaII bmyI mnlI    dsaV            apaLI/snoI
                                                 fnu4HI             asuI  apaLI/snoI   bstNI                  alw44I/snoI
                                 bsrI      munI  bbvI       mnlI   nlaIV  alw44I/snoI  apyI[dcm+]      draIII
2001 CGGCATTATT GGAGTGAAAA CCTTTTCCAG TGCTTCAATT GCAGCCTCTG CCTCAATGGG ACCGTGCACC TCTCCTGCCA GGAGAAACAG AACACCGTGT
     GCCGTAATAA CCTCACTTTT GGAAAAGGTC ACGAAGTTAA CGTCGGAGAC GGAGTTACCC TGGCACGTGG AGAGGACGGT CCTCTTTGTC TTGTGGCACA

                                                                                    hgiAI/aspHI
                                                                                    bsp1286
                                                                                    bsiHKAI
                                                                                    bmyI
                                                                              gsuI/bpmI
                                                                             scrFI
                                                                             mvaI   apaLI/snoI
                                                                             ecoRII
                                                                             dsaV
                 bspMI                                 scfI                  bstNI  alw44I/snoI
       bspMI nlaIII          ddeI                bsmAI      maeIII           apyI[dcm+]
2101 GCACCTGCCA TGCAGGTTTC TTTCTAAGAG AAAACGAGTG TGTCTCCTGT AGTAACTGTA AGAAAAGCCT GGAGTGCACG AAGTTGTGCC TACCCCAGAT
     CGTGGACGGT ACGTCCAAAG AAAGATTCTC TTTTGCTCAC ACAGAGGACA TCATTGACAT TCTTTTCGGA CCTCACGTGC TTCAACACGG ATGGGGTCTA

                                                            aluI
                                                          sstI
                                                          sacI
                                                          hgiJII
                                                          hgiAI/aspHI
                                                          ecl136II                                        bsp1286
                                  nlaIV                   bsp1286                                        nlaIV
                  bsp1286    pleI hgiCI                   bsiHKAI              maeIII          nlaIII    hgiCI
             tru9I       mnlI ddeI                        bmyI                hphI            nspI  dsaI banI
             mseI bmyI ddeI  hinfI banI                   banII          draIII               nspHI bsaJI bmyI
2201 TGAGAATGTT AAGGGCACTG AGGACTCAGG CACCACAGAC AAGAGAGTTG AGCTCAAAAC CCCACTTGGT GACACAACTC ACACATGCCC ACGGTGCCCA
     ACTCTTACAA TTCCCGTGAC TCCTGAGTCC GTGGTGTCTG TTCTCTCAAC TCGAGTTTTG GGGTGAACCA CTGTGTTGAG TGTGTACGGG TGCCACGGGT

                                              bsp1286
                                             nlaIV                                             bsp1286
     hgiJII                                  hgiCI    hgiJII                                  nlaIV    hgiJII
     bsp1286                             dsaI bmyI    bsp1286                            dsaI hgiCI    bsp1286
     bmyI                           bsp1286  banI     bmyI                               bsaJI bmyI    bmyI
     banII        maeIII   mnlI     bmyI bsaJI        banII        maeIII   mnlI    nlaIII     banI    banII
2301 GAGCCCAAAT CTTGTGACAC ACCTCCCCCG TGCCCACGGT GCCCAGAGCC CAAATCTTGT GACACACCTC CCCCATGCCC ACGGTGCCCA GAGCCCAAAT
     CTCGGGTTTA GAACACTGTG TGGAGGGGGC ACGGGTGCCA CGGGTCTCGG GTTTAGAACA CTGTGTGGAG GGGGTACGGG TGCCACGGGT CTCGGGTTTA
```

FIG. 6H

```
                                                                                   eam1105I
                                                                                 sau96I
                                                                        scrFI
                                         bsp1286                        mvaI     avaII
                                        nlaIV                           ecoRII
                                        hgiCI                           dsaV
                                    dsaI bmyI                           bstNI    asuI       mboII mboII
                                    bsaJI                               bsaJI mnlI         bpuAI earI/ksp632I       styI
         maeIII    mnlI    nlaIII    banI    alwNI                     apyI[dcm+]          bbsI mnlI               bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

              sau96I
              nlaIV
              avaII
              asuI            maeII
            mspI             pmlI
            hpaII            eco72I                                      sau96I
           scrFI      mnlI   bsaAI                           drdI        avaII              maeII
           nciI     ddeI maeIII                          mboII      mnlI                   bsaAI
           dsaV    eco81I    bbrPI                       bpuAI    bsaJI    bsrI           rsaI
           cauII   bsu36I/mstII/sauI        maeII        bbsI    avaI    asuI             csp6I        mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                     aciI
                    thaI
                    fnuDII/mvnI
                    bstUI
                    bsh1236I
                   sacII/sstII
                   nspBII                                                               scrFI
                   kspI                                                                 mvaI bsrI
                   dsaI                                                                 ecoRII
                   bsaJI                                                                dsaV
                   aciI                                               mnlI      econI   bstNI                rsaI
                  fnu4HI  mnlI             maeII                 hgaI     hphI     bslI    apyI[dcm+]           csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6I

```
                                                                                                    rsaI
                 bsmAI                                                                              csp6I
                bsaI            mnlI           taqI                                avaI           bsp1407I    bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                             aciI
          bsaJI                   bstNI               bstNI                              dsaI             nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                         bslI            fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI           bspMI                                   bsaJI           bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                        dsaI
        mspI                                   pleI                 mnlI            hphI                  fnu4HI
        hpaII                     mnlI  nlaIII hinfI     nlaIV   mboII   scfI     aluI  bsaJI       bspMI   bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                               scrFI
                                                                                                               nciI
                                                                                                               mspI
                                                                                                               hpaII
                                                                                                               dsaV
                                                                                         scrFI                 cauII
                                                                                         nciI                  bsaJI
                                                                                         mspI                haeIII/palI
                                    nlaIII                                               hpaII              mcrI
                                 ppu10I                            sapI                  dsaV               eagI/xmaIII/eclXI
         xmnI mboII              nsiI/avaIII                       mboII  mnlI       bsmAI                  eaeI
         asp700      nlaIII     sfaNI   mnlI           aciI        earI/ksp632I   bslI   cauII              cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6J

```
           rmaI
        mnlI
     sau3AI
     mboI/ndeII[dam-]                            styI
     dpnI[dam+]                               aciI
     dpnII[dam-]  taqI                       fnu4HI   sau96I
     alwI[dam-]  salI    scfI               sfiI ncoI haeIII/palI
    nlaIV  maeI  hincII/hindII              haeIII/palI
    bstYI/xhoII  accI    pstI              eaeI  dsaI asuI                 aluI
    bamHI xbaI pleI      bsgI    aluI cfrI  bsaJI                       fnu4HI
    alwI[dam-] hinfI bspMI     hindIII bglI nlaIII                      bbvI          maeIII          sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]
                                                                                                    dpnII[dam-]
                                                                                                   pvuI/bspCI
                                                                                                   mcrI
                                                                                                 taqI[dam-]    tru9I
                                                                                                clal/bsp106[dam-]
                                                                                               sau3AI        mseI
                                                                                               mboI/ndeII[dam-]
                                                                                               dpnI[dam+] xmnI        hinPI
                                            rmaI                                               dpnII[dam-]  aseI/asnI/vspI
                                      bsmI  maeI                                      nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                      rsaI
                                                      csp6I
         haeI                                        nlaIV
       styI                                          kpnI
       ncoI                                          hgiCI
       dsaI haeIII/palI                              banI                     aluI
     fnu4HI nlaIII                                   asp718     mnlI         pvuII
     bbvI  bsaJI          mnlI      mnlI             acc65I   ddeI aciI      nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6K

```
                 nlaIV                                                                          nlaIV
             scrFI                                                  scrFI        scrFI
             mvaI                                 ppu10I                  mvaI               mvaI
             ecoRII                      nsiI/avaIII                ecoRII       ecoRII
             dsaV                       nlaIII                      dsaV         dsaV
             bstNI                          sphI                          bstNI              bstNI
             apyI[dcm+]                     nspI sfaNI                    apyI[dcm+]         apyI[dcm+]
            bsaJI                           nspHI                        sexAI             bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                     sfaNI
                   ppu10I
                   nsiI/avaIII
                  nlaIII
                 sphI
                 nspI                                                      aciI                                bslI
                 nspHI                              aciI         aciI  fokI             aciI bsrI  aciI        aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC
                                                                                                              rmaI
                                                                                                             styI
                                                                                                             bsaJI
                                                   fnu4HI                                                    blnI
        nlaIII                                    sfiI mnlI                                                  avrII
       styI                                       haeIII/palI                                              haeIII/palI
       ncoI                                  bsaJI bglI          ddeI                                     stuI
       dsaI                                haeIII/palI bsaJI  mnlI aluI                    mnlI           haeI
       bsaJI                             mnlI mnlI aciI     haeIII/palI                 mnlI             mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                              scrFI
                                                                              mvaI
                                                                              ecoRII
                       tru9I             haeIII/palI                          dsaV
                      hpaI              eaeI                                  bstNI
                      hincII/hindII     cfrI                      bsrI        apyI[dcm+]       tru9I       fnu4HI
                  aluI mseI  aluI     bsrI               maeII maeIII        bsaJI   maeIII     mseI        bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6L

```
                                                                                                                  hinPI
                                                                                                                  hhaI/cfoI
                                                                                                                 nlaIV
                                                                                                                 narI
                                                         sau3AI                                                  kasI
                                             sau96I      mboI/ndeII[dam-]                                        hinlI/acyI
                                             haeIII/palI                                                         hgiCI
                                             asuI        dpnI[dam+]                                              haeII      aciI
                     aluI                  mnlI          dpnII[dam-]                                             banI    sfaNI
                    pvuII               mboII    aciI   pvuI/bspCI                                               ahaII/bsaHI
     fokI           nspBII              earI/ksp632I    mcrI                              bglI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                                   aciI
                                                                                                                fnu4HI
                                                                            hinPI                               aciI
                                                                           thaI                                thaI
                                                                           fnuDII/mvnI                         fnuDII/mvnI
                                                                           bstUI scfI      hinPI               bstUI
                                                                           bsh1236I        hhaI/cfoI hinPI
                                                                       rsaI hhaI/cfoI  fnu4HI    tru9I bsh1236I
                        sfaNI    aciI          aciI   maeII            csp6I    bslI    aciI     mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

           fnu4HI
         hinPI
         hhaI/cfoI                             hinPI
        thaI                                   hhaI/cfoI
        fnuDII/mvnI                          rmaI                                                               mspI
        bstUI                           hinPI  haeII                                                            hpaII
        bsh1236I     aciI               hhaI/cfoI    bsrBI                                                     naeI
     maeIII bbvI  maeIII               haeII maeI    aciI             mboII                             maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                      nlaIV
                     hgiJII
                     bsp1286                             nlaIV                                                 maeII    haeIII/palI
                     bmyI                                hgiCI   taqI                                         draIII   sau96I
      aluI           banII         nlaIV                 banI mnlI                              hphI          bsaAI    asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6M

```
                                       maeII pleI           tru9I    pleI                            bslI
                                      drdI   hinfI  maeII   mseI     hinfI          bsrI            bslI  avaI
4201 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG
     TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

                                                                                             thaI
                                                                                             fnuDII/mvnI
                                                         tru9I                         tru9I   apoI tru9I      tru9I
                                                         mseI              tru9I       mseI bstUI   mseI       mseI
                                              haeIII/palI         aluI     mseI    apoI    bsh1236I        sspI
4301 GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCGG CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
     CCGATAAGAA AACTAAATAT TCCCTAAAAC GGCTAAAGCC GGATAACCAA TTTTTTACTC GACTAAATTG TTTTTAAATT GCGCTTAAAA TTGTTTTATA

                         hgiAI/aspHI
                         bsp1286
                         bsiHKAI                                                                              hinPI
                         bmyI    ddeI                   aciI                                   maeIII         fnu4HI
       maeII             apaLI/snoI   rsaI             fnu4HI    tru9I                      maeII bsrI    nlaIII hhaI/cfoI
      psp1406I           alw44I/snoI  csp6I        sfaNI         mseI        aciI        bsaAI tth111I/aspI bbvI
4401 TAACGTTTAC AATTTTATGG TGCACTCTCA GTACAATCTG CTCTGATGCC GCATAGTTAA GCCAACTCCG CTATCGCTAC GTGACTGGGT CATGGCTGCG
     ATTGCAAATG TTAAAATACC ACGTGAGAGT CATGTTAGAC GAGACTACGG CGTATCAATT CGGTTGAGGC GATAGCGATG CACTGACCCA GTACCGACGC

                                                                                                  scrFI
                                  hinPI                          sfaNI                            nciI
                                  hhaI/cfoI                   mspI                                mspI
                                 thaI                         hpaII                               hpaII     nspI
                                 fnuDII/mvnI                 scrFI                                dsaV      nspHI
                                 bstUI                       nciI                             esp3I      fnu4HI
                         nspBII  bsh1236I                    dsaV fokI                  maeIII bsmAI     bbvI
              aciI       aciI  hgaI      drdI                cauII   aciI          aluI    bslI   cauII aluI nlaIII
4501 CCCCGACACC CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT
     GGGGCTGTGG GCGGTTGTGG GCGACTGCGC GGGACTGCCC GAACAGACGA GGGCCGTAGG CGAATGTCTG TTCGACACTG GCAGAGGCCC TCGACGTACA

                                        thaI
                                        fnuDII/mvnI
                                        bstUI
                                        bsh1236I
                                       hinPI
                                       hhaI/cfoI                      mnlI
                                    thaI                            haeIII/palI
                                    fnuDII/mvnI         mboII      sau96I                             nlaIII
                                    bstUI mnlI          bpuAI      asuI                           tru9I  rcaI
         mnlI    hphI       hphI    bsh1236I            bbsI      eco0109I/draII                  mseI   bspHI
4601 GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA GTATTCTTGA AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA ATGTCATGAT
     CAGTCTCCAA AAGTGGCAGT AGTGGCTTTG CGCGCTCCGT CATAAGAACT TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT TACAGTACTA
```

FIG. 6N

```
                                                          nlaIV
                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                hinlI/acyI                             bstUI                                                               rcaI
                ahaII/bsaHI                            bsh1236I                                                            bspHI
                aatII                                 hinPI                                                            bsrBI
            ddeI maeII                                hhaI/cfoI                                                        aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                     mboII                                                           fnu4HI
  bsmAI                                   sspI       earI/ksp632I                                                    aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                               hgiAI/aspHI
                                                                               bsp1286
                                                                     sau3AI    bsiHKAI                         sau3AI
                                                                     mboI/ndeII[dam-]                          mboI/ndeII[dam-]
                                                                     dpnI[dam+] bmyI                           dpnI[dam+]
                                                                     dpnII[dam-]                              bstYI/xhoII
                                                                eco57I         apaLI/snoI                  bsrI dpnII[dam-]
                          hphI            hphI            sfaNI  mboII[dam-]   alw44I/snoI  maeIII taqI     alwI[dam-]
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                          aciI
        sau3AI                                                                           thaI
        mboI/ndeII[dam-]                    maeII                                        fnuDII/mvnI
        dpnI[dam+]                         psp1406I          hgiAI/aspHI                 bstUI
        dpnII[dam-]                        xmnI              bsp1286 tru9I               bsh1236I           hinlI/acyI
  aciI  alwI[dam-]                         asp700            bsiHKAI mseI               hinPI              hgaI
nspBII  bstYI/xhoII                      mboII               bmyI   ahaIII/draI         hhaI/cfoI          ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

 scrFI
 nciI
 mspI
 hpaII                                                              rsaI
 dsaV                      aciI                                     csp6I   bsrI
 cauII      bcgI  mcrI  fnu4HI         ddeI                        scaI  hphI  maeIII      sfaNI     fokI    nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 60

```
                                                                                             sau96I
                                                                                             avaII
                                                                                     sau3AI  asuI
                                                       haeIII/palI                   mboI/ndeII[dam-]
                                                        eaeI                         dpnI[dam+]
                                                        cfrI                         dpnII[dam-]
                fnu4HI                                  fnu4HI                       pvuI/bspCI
                bbvI        nlaIII                      aciI                         mcrI    mnlI          aluI    aciI
5201 TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA CCGCTTTTTT
     ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT GGCGAAAAAA

                  maeIII
               nlaIII
            sau3AI                                    mspI
            mboI/ndeII[dam-]    sau3AI  nlaIV
            dpnI[dam+]          mboI/ndeII[dam-} aluI
            dpnII[dam-]         dpnI[dam+]    hpaII                                                           fnu4HI
        nlaIII alwI[dam-]       dpnII[dam-} bsaWI                                        maeIII     sfaNI    bbvI
5301 GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT GCCAGCAGCA
     CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA CGGTCGTCGT

                                                                  mspI
             hinPI                                                hpaII
            mstI                                                 scrFI
            aviII/fspI     bsrI                         aluI     nciI        tru9I      fokI                       sau96I
       maeII hhaI/cfoI    tru9I                        rmaI      dsaV        mseI     bsrI          aciI           avaII
      psp1406I            mseI                         maeI      cauII      aseI/asnI/vspI   mnlI                  asuI
5401 ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA GTTGCAGGAC
     TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT CAACGTCCTG

             bglI                                                  mspI             thaI
            sau96I                                                 hpaII            fnuDII/mvnI              haeIII/palI
            haeIII/palI                                           cfr10I            bstUI                   sau96I
       hinPI  asuI      mspI                                   nlaIV hphI        bsmAI aciI       fnu4HI    nlaIV
       hhaI/cfoI        hpaII                               gsuI/bpmI           bsaI  bsh1236I    bbvI bsrI asuI
5501 CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC TGGGGCCAGA
     GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG ACCCCGGTCT

                                                                                          ddeI
                                                                                      sau3AI      nlaIV
                                               pleI                                   mboI/ndeII[dam-]  mnlI
                                               hinfI                                  dpnI[dam+]    hgiCI       tru9I
         mnlI                               eam1105I           fokI                   dpnII[dam-]   banI        mseI
5601 TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC CTCACTGATT
     ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG GAGTGACTAA
```

FIG. 6P

```
                                                                                     rmaI       sau3AI
                                                                                 sau3AI hphI    mboI/ndeII[dam-]
                                                                                 mboI/ndeII[dam-]
                                                                                 dpnI[dam+]     dpnI[dam+]
                                                                                 dpnII[dam-]    dpnII[dam-]
                                                        tru9I            ahaIII/draI   maeI     alwI[dam-]
                                                        mseI          tru9I tru9I bstYI/xhoII  bstYI/xhoII
            maeIII                                     ahaIII/draI    mseI  mseI  alwI[dam-] mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                sau3AI
                                                                                mboI/ndeII[dam-]
                                                                                dpnI[dam+]   sau3AI
                                                                                dpnII[dam-]  mboI/ndeII[dam-]
                                                                               bstYI/xhoII   dpnI[dam+]
                                                                        sau3AI alwI[dam-]    dpnII[dam-]
            nlaIII                maeII                                 mboI/ndeII[dam-]     alwI[dam-]
           rcaI                tru9I                    hgaI            dpnI[dam+] mboII[dam-]
           bspHI               mseI                 ddeI                dpnII[dam-]         bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                  sau3AI
      thaI                                                        mboI/ndeII[dam-]
      fnuDII/mvnI                                                 dpnI[dam+]
      bstUI                                                       dpnII[dam-]
      bsh1236I                                                   alwI[dam-]
     hinPI     fnu4HI                           aciI          mspI                                  bsrI
     hhaI/cfoI bbvI                   aciI     nspBII         hpaII      aluI                    maeIII   eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

              hinPI                  rmaI                 haeIII/palI
              hhaI/cfoI              maeI     bslI       haeI                scfI   aciI       mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6Q

```
                                                        scrFI                                  aciI
                                                        nciI                                 nspBII
                      fnu4HI                            mspI                       mspI     fnu4HI
              alwNI   bbvI                              hpaII                      hpaII    bbvI
             bsrI  fnu4HI                               dsaV     pleI             bsaWI    hinPI mcrI
        maeIII     bbvI    bsrI                         cauII    hinfI           maeIII    hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

              hgiAI/aspHI
              bsp1286
              bsiHKAI
              bmyI                                                                           hinPI
              apaLI/snoI                                                                     hhaI/cfoI
              alw44I/snoI  aluI                           ddeI      scfI                    haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                            scrFI
                                                                            mvaI         scrFI
                                                                            ecoRII  mvaI
                          mspI                                              dsaV    ecoRII
                          hpaII                                             bstNI        dsaV
                          bslI   fnu4HI                      hinPI mnlI     bsaJI        bstNI
           aciI          bsaWI   aciI                        hhaI/cfoI aluI apyI[dcm+]   apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                              haeIII/palI
                                                                                             fnu4HI
                                                                                             aciI
                                                                                            thaI bslI
                                                                                            fnuDII/mvnI
                                 taqI                                   nlaIV               bstUI
               mnlI drdI     hgaI            sfaNI                  aciI                    bsh1236I     nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6R

```
        haeIII/palI
       haeI
     scrFI
     mvaI bslI
     ecoRII                 nlaIII
     dsaV                  nspI
     bstNI        haeIII/palI  nspHI                   tfiI                                          bsrBI
     apyI[dcm+]  haeI          aflIII                  hinfI                  aciI          aluI     aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                       thaI
                                                                                       fnuDII/mvnI
                                                                                       bstUI
                                                                                       bsh1236I
                                                                                      hinPI
                                                                                      hhaI/cfoI
                                                                                     thaI
                                                                                     fnuDII/mvnI
       fnu4HI             fnu4HI                 sapI  hinPI                         bstUI   haeIII/palI
       bbvI               bbvI pleI              mboII hhaI/cfoI                     bsh1236I         tru9I
      aciI               hinPI  hinfI            earI/ksp632I           mnlI     bslI     eaeI  tfiI aseI/asnI/vspI
     fnu4HI     mcrI     hhaI/cfoI      mnlI   aciI   haeII           aciI      aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA
                                                                                           scrFI
                                                                                           mvaI
                                                                                           ecoRII
                                                                                           dsaV
       aluI                                                      tru9I                nlaIV bstNI
      pvuII                                        hinPI         mseI      maeIII     hgiCI apyI[dcm+]
      nspBII                 bsrI     aciI         hhaI/cfoI   aseI/asnI/vspI  mnlI   banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                        tru9I
                                                                                        mseI
                                                                                       aseI/asnI/vspI
                mspI                        aciI                                      xmnI
                hpaII                     bsrBI                 aluI      nlaIII      asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT
```

>length: 6889

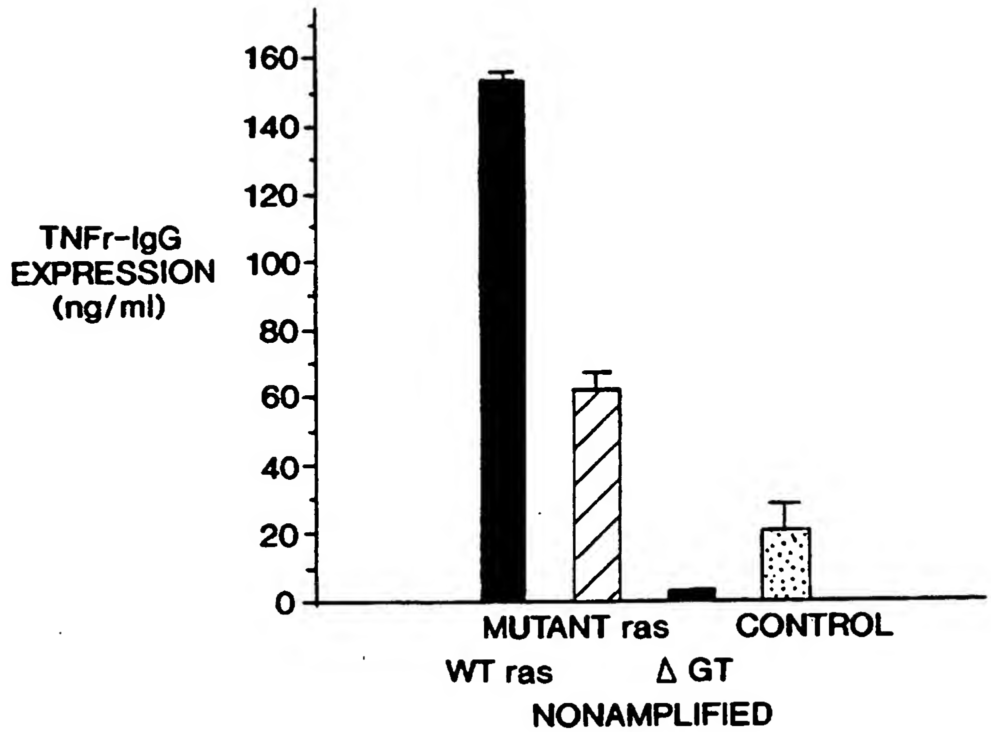
TNFr-IgG
EXPRESSION
(ng/ml)
160
140
120
100
80
60
40
20
0
MUTANT ras
CONTROL
WT ras
Δ GT
NONAMPLIFIED

FIG. 7A

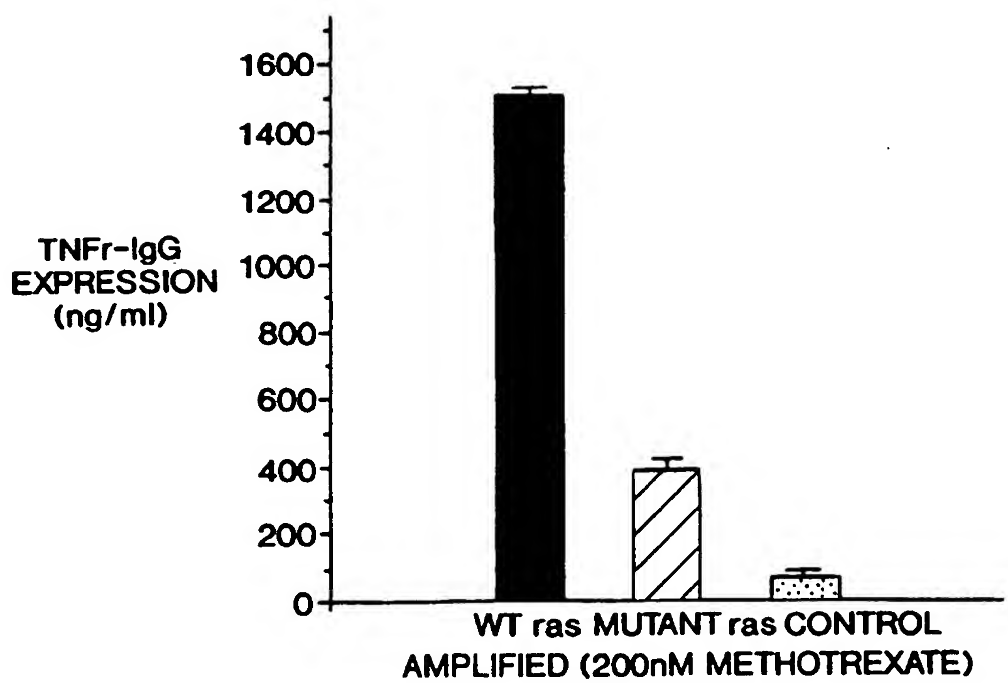
TNFr-IgG
EXPRESSION
(ng/ml)
1600
1400
1200
1000
800
600
400
200
0
WT ras MUTANT ras CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 7B

FIG. 8

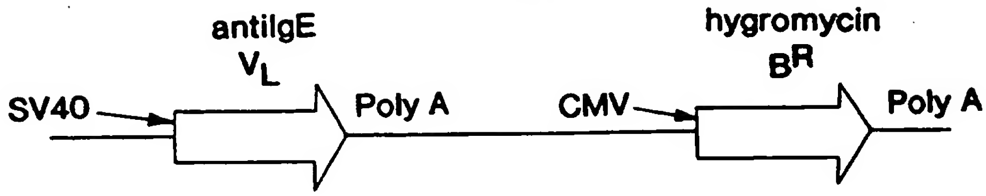
antiIgE
V_L
SV40
Poly A
CMV
hygromycin
B^R
Poly A

FIG. 11

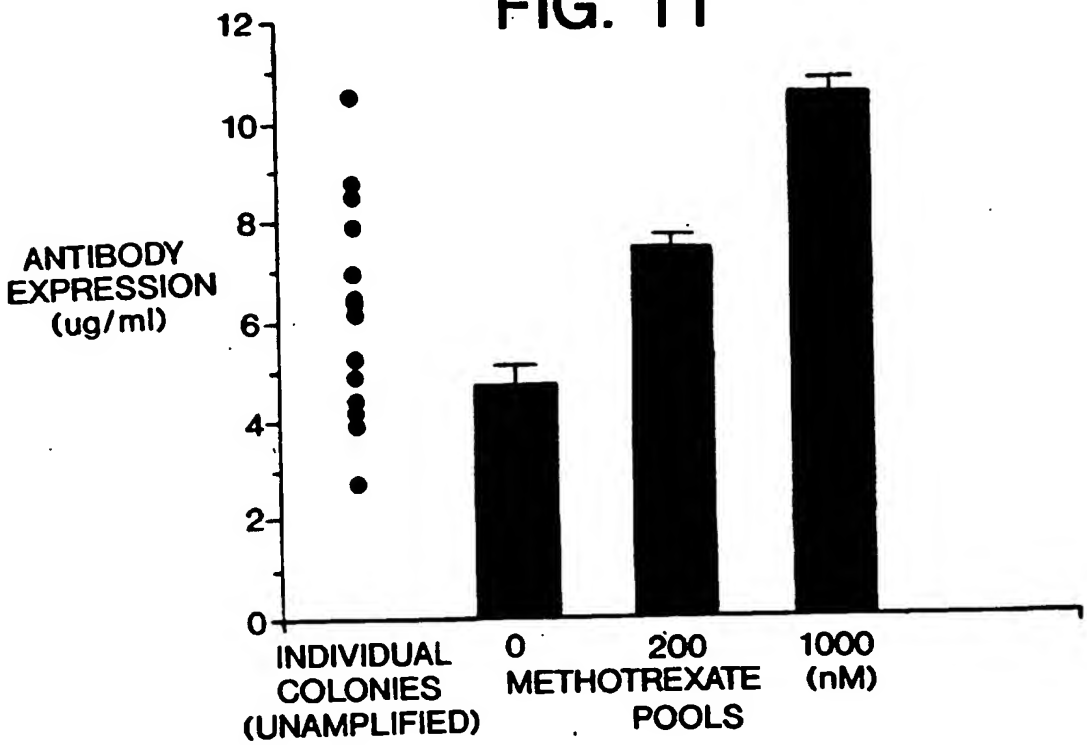
ANTIBODY EXPRESSION (ug/ml)
12
10
8
6
4
2
0
INDIVIDUAL COLONIES (UNAMPLIFIED)
0
200
1000
METHOTREXATE POOLS
(nM)

FIG. 9A

```
   aluI
   sstI
   sacI
   hgiJII
   hgiAI/aspHI
   ecl136II
   bsp1286
   bsiHKAI
   bmyI                                    aluI                                                          nlaIV
   banII                         sau3AI pvuII                                                          scrFI
  taqI                           mboI/ndeII[dam-]                                                      mvaI
                                 dpnI[dam+]                                                            ecoRII
                                pvuI/bspCI                                                             dsaV
                             pleI dpnII[dam-]                                                          bstNI
                             hinfI  taqI[dam-]                                                         apyI[dcm+]
                          rmaI   mcrI    nspBII                                                       bsaJI
                          maeI  taqI[dam-]
1  TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
   AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                              nlaIV
           sfaNI                     scrFI      scrFI                                  sfaNI
          ppu10I                     mvaI           mvaI                                      ppu10I
          nsiI/avaIII                ecoRII     ecoRII                                nsiI/avaIII
         nlaIII                      dsaV       dsaV                                 nlaIII
        sphI                         bstNI          bstNI                                    sphI
        nspI                         apyI[dcm+]     apyI[dcm+]                               nspI
        nspHI                       sexAI          bsaJI                                     nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                  nlaIII
                                                                                 styI
                                                                                 ncoI
                                         aciI                                 bslI dsaI
            aciI              aciI  fokI                   aciI bsrI  aciI    aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                       rmaI
                                                                      styI
                                                                      bsaJI                          haeIII/palI
            fnu4HI                                                    blnI                          mcrI
            bglI                                                      avrII            aluI         eagI/xmaIII/eclXI
           sfiI                                                     haeIII/palI      rmaI           eaeI
           haeIII/palI                                             stuI              maeI           cfrI
         mnlI    mnlI       ddeI                                   haeI             nheI           mspI
      haeIII/palI bsaJI  mnlI    aluI               mnlI           mnlI maeI        aluI           hpaII
     mnlI bsaJI aciI    haeIII/palI               mnlI
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 9B

```
                              tfiI
    scrFI                     hinfI
    nciI                   aciI
    mspI                  thaI                                                                    fnu4HI
    hpaII                 fnuDII/mvnI                        aciI                                bbvI
    dsaV                  bstUI                     maeII  rsaI                                 nspBII
    cauII                 bsh1236I               maeIII    csp6I  scfI            mnlI          aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                              haeIII/palI
                                                                             haeI
                                                                           scrFI
                                                                           mvaI     bsrBI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI    aciI                 rsaI
                                     pflMI                       bsmAI     apyI[dcm+]        xmnI         csp6I
    taqI   .       sfaNI             bslI                        bsaI     bsaJI  mnlI  ddeI  asp700      scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                        eco57I                                            dsaV
                       mboII                 tfiI                         bstNI                   tfiI       tru9I
                      earI/ksp632I           hinfI                        apyI[dcm+]              hinfI      mseI
                     mnlI                  alwNI    hphI                 sexAI            ddeI mboII taqI  ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
             tru9I                                          bmyI                                  tru9I
             mseI         ddeI                              banII                                aflII/bfrI
            aseI/asnI/vspI                       bslI   mnlI aluI          bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9C

```
                                                                                              haeIII/palI
                                                                                             haeI
                                                                      scrFI         scrFI
                                                                      mvaI                poaI
                                                                      ecoRII        ecoRII
       mspI                                                           dsaV    tfiI  dsaV
       hpaII                                                          bstNI  nlaIII       bstNI     ddeI pleI
      bsaWI                 accI nlaIII         mnlI                  apyI(dcm+)  hinfI   apyI(dcm+)     hinfI
801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
    TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                hgaI
                                                                                               hinlI/acyI
                                                                                               ahaII/bsaHI
                                                                                            scrFI
            nlaIII                                                                          mvaI     mnlI
        sau3AI                                                                              ecoRII
        mboI/ndeII(dam-)                                                                    dsaV
        dpnI(dam+)            maeII                                                         bstNI    ecoNI
        dpnII(dam-)          aflIII                                                         apyI(dcm+)    mnlI
    maeIII alwI(dam-)  apoI      maeIII                                          mnlI      bsaJI    bslI ddeI
901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
    CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

        scrFI
        mvaI
        ecoRII
        dsaV
        bstNI
        apyI(dcm+)
     sau96I
     avaII                                                              sfaNI
     asuI   mnlI     sfaNI                  accI     mboII                 mboII                      mnlI     aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                   styI
                                                   bsaJI
                                             sau3AI
                    nlaIII                   mboI/ndeII(dam-)
                   styI                      dpnI(dam+)
                   ncoI                      dpnII(dam-)          aluI     tru9I
     ppu10I        dsaI                      alwI(dam-)         fnu4HI    mseI
     nsiI/avaIII   bsaJI                    bstYI/xhoII         bbvI     aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9D

```
                                                                                      sau96I
                                                                                      avaII
                                                                                      asuI
                                                                                   scrFI
                                                                                   mvaI
                                                                                   ecoRII
                                                                                   dsaV
                                                                                   bstNI                                ecoRI
                maeIII                                                             apyI[dcm+]        mnlI        taqI   apoI
               hphI    scfI        fokI                                    bslI   bsaJI              bsaJI      claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                        scrFI
       nlaIII                                                                                           mvaI
      styI                                                                                              ecoRII
      pflMI                                                                                             dsaV
      ncoI                                                                                              bstNI fnu4HI
      dsaI                                                         rsaI              aluI               apyI[dcm+]
      bslI fokI                            rmaI              gsuI/bpmI              pvuII      pleI        haeI     bbvI
      bsaJI       nlaIII  fokI             maeI             bsrI   csp6I            nspBII     hinfI    aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9E

```
                                                                                                   scrFI
                                                                                                   nciI
                                                                                                   mspI
                                                                                                   hpaII
                                                                                                   dsaV
                                                                                                   cauII
                                                                                                   bslI
                                                                                                  xmaI/pspAI
                                                                                                  smaI
                                                                                                  scrFI
                                                                                                  nciI
                                                                                                  dsaV
                                                                                                  cauII        scrFI
           hgiJII                                                                              sau96I          mvaI
           bsp1286                                                                   sau3AI    nlaIV           ecoRII
           bmyI                                                 mspI                 mboI/ndeII[dam-]          dsaV
      scrFI                                               hpaII                dpnI[dam+]   bslI         bstNI
      mvaI banII                                                 mroI                 dpnII[dam-]  bsaJI        bslI
      ecoRII                                             bspMII               nlaIV      haeIII/palI     apyI[dcm+]
      dsaV                                               bspEI                bstYI/xhoII   avaI      sau96I
      bstNI                                                      bsaWI                bamHI     eco0109I/draII haeIII/palI
      bsaJI                                                  mnlI                   alwI[dam-] asuI         asuI
      apyI[dcm+]                     alwNI               hphI  accIII    aluI   bsrI alwI[dam-]  bsaJI     eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                   sau3AI
                                   mboI/ndeII[dam-]
                                   dpnI[dam+]                                           thaI
                        maeII      dpnII[dam-]                                          fnuDII/mvnI
                  taqI snaBI      alwI[dam-]               hgaI     haeIII/palI         bstUI  tfiI
                 claI/bsp106    mspI                    xmaI       sau96I               bsh1236I
              sfaNI    bsaAI    hpaII taqI[dam-]        maeI       asuI                nruI     hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9F

```
                                                                                                                     scrFI
                                                                                                                     mvaI
                                                                                                                     ecoRII
                                                                                                                     dsaV
                                                                                                                     bstNI
                                                                                                                     apyI[dcm+]
                                                                                                                  hinPI
                                                                                                                  hhaI/cfoI
                                                                                  mnlI                           nlaIV
                                                                               xhoI                              narI
                                                                               paeR7I                            kasI
                                                                               avaI                              hinlI/acyI
         scfI                                                               hgiAI/aspHI                          hgiCI
         pstI                                                               bsp1286                              haeII
         bsgI                        mnlI                                   bsiHKAI    fnu4HI                    banI
       bspMI                      ddeI drdI                                 bmyI taqI bbvI                       ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                             sau96I
                                             haeIII/palI
                                            sau96I
                                            nlaIV                                                                         scrFI
                                            hgiJII                        nlaIV                                           mvaI
                                            bsp1286                       hgiCI                                           ecoRII
             scrFI                     bsp120I                      banI                                                  dsaV
             mvaI                          bmyI                        scrFI                                         bstNI
             ecoRII                    banII                     mvaI                                                bsaJI
             dsaV                      asuI                      ecoRII                                            sau96I
             bstNI hphI                    apaI                mboII   dsaV              hgiAI/aspHI           haeIII/palI
             apyI[dcm+] bsmAI     haeIII/palI ecoO109I/draII           bstNI                   bsp1286               asuI
           bsaJI maeIII       mnlI          styI asuI      bpuAI    apyI[dcm+]  mnlI          bsiHKAI        fnu4HI
       nlaIV   bstEII esp3I bsaJI mnlI    bsaJI         bbsI     bsaJI       mnlI          bmyI mnlI    bsp1286 aciI apyI[dcm+]
                                                                                                        bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9G

```
          scrFI                                            hinPI
          mvaI                                                  nlaIV
          ecoRII                                          narI
          ecoNI                                                 kasI                hgiAI/aspHI
          dsaV                phI                     hinlI/acyI          bsp1286      mspI
          bstNI                   mspI                      hgiCI               bsiHKAI      hpaII
          bslI                    hpaII                     haeII               bmyI        scrFI
          apyI[dcm+]            cfr10I                      banI          fnu4HI      nciI
      fnu4HI                    bsaWI  tth111I/aspI         ahaII/bsaHI   aciI  apaLI/snoI  dsaV
      bbvI                bslI  ageI maeIII             ddeI hhaI/cfoI  nspBII  alw44I/snoI cauII      scfI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                               nlaIV
      ddeI pleI                                       fnu4HI   hgiCI
     mnlI  hinfI                               mnlI   bbvI     banI                        tfiI
     eco81I           mnlI  fnu4HI  maeIII bsp1286 rmaI      bsp1286                    hinfI                styI
     bsu36I/mstII/sauI ddeI bbvI   hphI    bmyI    maeI aluI  bmyI                     maeII                bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                                eam1105I
                                                                                               sau96I
                                                                                       scrFI
                                                                                       mvaI    avaII
                                                                                       ecoRII
                         hgiJII                                                        dsaV
                         bsp1286                        nlaIII                         bstNI   asuI       mboII mboII
                         bmyI                          nspI        bsp1286             bsaJI  nlaIV      bpuAI earI/ksp632I
                         banII          maeIII         nspHI       bmyI  alwNI         apyI[dcm+]        bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9H

```
                                                  sau96I
                                                  nlaIV
                                                  avaII
                                                mspI
                                                scrFI
                                                nciI
                                           sau3AI hpaII
                                           mboI/ndeII[dam-]
                                           dpnI[dam+]                          nlaIII
                                        nlaIII    dsaV        mnlI           nspI                                    drdI   mnlI
                                       rcaI       cauII      ddeI maeIII                                         mboII    ddeI
                      styI             bspHI[dam-]   asuI   eco81I      nspHI                                     bpuAI    eco81I
                      bsaJI          mnlI  dpnII[dam-]   bsu36I/mstII/sauI                     maeII             bbsI     bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                       aciI
                                                      thaI
                                                      fnuDII/mvnI
                                                      bstUI
                                                      bsh1236I
                                                     sacII/sstII
                                                     nspBII
                                                     kspI
       maeII                                         dsaI                                 rsaI
     rsaI                                            bsaJI                                csp6I
     csp6I                                           aciI                 rsaI        maeII                          mnlI        ecoNI
  bsrI bsaAI                 mnlI                   fnu4HI      mnlI      csp6I      bsaAI                  hgaI      hphI       bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

    scrFI
    mvaI bsrI
    ecoRII
    dsaV
    bstNI                   rsaI            bsmAI                                                                 fnu4HI
    apyI[dcm+]              csp6I         bsaI              mnlI              taqI                                 bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9I

```
                                           scrFI
                                           nciI
                                           mspI
                                           hpaII
                                           dsaV
                                           cauII
                                          xmaI/pspAI
                                          smaI
                                          scrFI                   scrFI
                                          nciI                    mvaI                scrFI
                                          dsaV                    ecoRII        mvaI
                                          cauII                   dsaV          ecoRII
                       rsaI            fokI    mboII              bstNI               dsaV
                       csp6I        bslI  bsaJI                   apyI[dcm+]          bstNI
      avaI            bsp1407I     bslI   avaI earI/ksp632I      sexAI          bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

           dsaI                         mspI
           bslI                    fnu4HI                                      pleI                  mnlI
           bsaJI                   bbvI hpaII                     mnlI         hinfI     nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                             bpuAI
                                             bbsI                   nlaIII
          dsaI                             maeII                ppu10I                            sapI
          bsaJI                fnu4HI    xmnI mboII             nsiI/avaIII                       mboII  mnlI
       hphI            bspMI   bbvI      asp700      nlaIII     sfaNI   mnlI                      earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                   styI
           scrFI                           taqI                 aciI
           nciI                           salI                 fnu4HI   sau96I
           mspI                     rmaI  hincII/hindII       sfiI ncoI haeIII/palI
           hpaII                    maeI  accI   scfI         haeIII/palI
           dsaV                sau96I  pleI     pstI        eaeI  dsaI asuI                 aluI
           cauII               haeIII/palI      bsgI   aluI cfrI  bsaJI                  fnu4HI
       bsmAI                   asuI    hinfI bspMI   hindIII bglI nlaIII                 bbvI           maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9J

```
                                                                                                   taqI[dam-]
                                                                                                  claI/bsp106[dam-]
                                                                                                 sau3AI
                                                                                                 mboI/ndeII[dam-]
                                                                                                 dpnI[dam+]
                                                             rmaI                                dpnII[dam-]
              sfaNI    apoI                           bsmI   maeI                       nlaIII   alwI[dam-]
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

               tru9I                                                   rsaI
           xmnI                                                        csp6I
      sau3AI  aseI/asnI/vspI      styI                                nlaIV
      mboI/ndeII[dam-]            ncoI                                kpnI
      dpnI[dam+]            fnu4HI    haeI                            hgiCI
      dpnII[dam-]           bbvI  dsaI haeIII/palI                    banI                           aluI
     pvuI/bspCI mseI     hinPI      bsaJI                             asp718     mnlI               pvuII
     mcrI   asp700       hhaI/cfoI nlaIII         mnlI       mnlI     acc65I   ddeI aciI            nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                  nlaIV
                               scrFI                                                    scrFI
                               mvaI                           ppu10I                        mvaI
                               ecoRII                     nsiI/avaIII                   ecoRII
                               dsaV                      nlaIII                         dsaV
                               bstNI                        sphI                            bstNI
                               apyI[dcm+]                   nspI sfaNI                      apyI[dcm+]
                              bsaJI                         nspHI                          sexAI         bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

        nlaIV                             sfaNI
     scrFI                        ppu10I
     mvaI                              nsiI/avaIII
     ecoRII                      nlaIII
     dsaV                       sphI
     bstNI                            nspI                                                      aciI
     apyI[dcm+]                       nspHI                       aciI         aciI   fokI             aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9K

```
                                                                        fnu4HI
                              nlaIII                                    sfiI mnlI
                             styI                                       haeIII/palI
                             ncoI                                  bsaJI bglI          ddeI
                       bslI  dsaI                                haeIII/palI bsaJI  mnlI  aluI                   mnlI
     bsrI  aciI        aciI  bsaJI                             mnlI  mnlI aciI    haeIII/palI                 mnlI
3201 CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG
     GGTCAAGGCG GGTAAGAGGC GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC

                                                          fnu4HI            hinPI
                      rmaI                              mcrI                hhaI/cfoI
                     styI                               eagI/xmaIII/eclXI  thaI
                     bsaJI                              eaeI               fnuDII/mvnI
                     blnI                           bsrBI          tru9I   bstUI               bspMI
                     avrII                        xhoI notI    tru9I       bsh1236I         scfI
                   haeIII/palI                    paeR7I haeIII/palI     hinPI     tru9I    pstI
                  stuI                            avaI fnu4HI  pacI      hhaI/cfoI mseI     bsgI
                  haeI                       maeIII taqI cfrI  mseI      bssHII   ahaIII/draI   maeIII
                 mnlI maeI              aluI     mnlI  aciI aciI   mseI ascI     swaI    sse8387I     aluI    bsrI
3301 AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AAAGCTGTTA CCTCGAGCGG CCGCTTAATT AAGGCGCGCC ATTTAAATCC TGCAGGTAAC AGCTTGGCAC
     TCCGAAAAAA CCTCCGGATC CGAAAACGTT TTTCGACAAT GGAGCTCGCC GGCGAATTAA TTCCGCGCGG TAAATTTAGG ACGTCCATTG TCGAACCGTG

                                           scrFI
                                           mvaI
                                           ecoRII
                                           dsaV
      haeIII/palI                          bstNI                                                aluI
     eaeI                      bsrI        apyI[dcm+]        tru9I       fnu4HI                pvuII            mboII
     cfrI             maeII maeIII        bsaJI   maeIII     mseI        bbvI  fokI            nspBII           earI/ksp632I
3401 TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCCTTC GCCAGCTGGC GTAATAGCGA
     ACCGGCAGCA AAATGTTGCA GCACTGACCC TTTTGGGACC GCAATGGGTT GAATTAGCGG AACGTCGTGT AGGGGGGAAG CGGTCGACCG CATTATCGCT

                                                                hinPI
                                                                hhaI/cfoI
                                                               nlaIV
                   sau3AI                                      narI
                   mboI/ndeII[dam-]                            kasI
                   dpnI[dam+]                                  hinlI/acyI
        sau96I     dpnII[dam-]                                 hgiCI
        haeIII/palI                                            haeII      aciI
        asuI      pvuI/bspCI                                   banI    sfaNI
      mnlI aciI   mcrI                        bglI             ahaII/bsaHI                    sfaNI   aciI
3501 AGAGGCCCGC ACCGATCGCC CTTCCCAACA GTTGCGTAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA
     TCTCCGGGCG TGGCTAGCGG GAAGGGTTGT CAACGCATCG GACTTACCGC TTACCGCGGA CTACGCCATA AAAGAGGAAT GCGTAGACAC GCCATAAAGT
```

FIG. 9L

```
                                                          aciI
                   hinPI                                fnu4HI                 fnu4HI
                   hhaI/cfoI                            aciI                 hinPI
                  thaI                                 thaI                  hhaI/cfoI
                  fnuDII/mvnI                          fnuDII/mvnI          thaI
                  bstUI                hinPI           bstUI                fnuDII/mvnI
                  bsh1236I             hhaI/cfoI hinPI                      bstUI                               hinPI
               rsaI      scfI fnu4HI     tru9I bsh1236I                    bsh1236I    aciI                 hhaI/cfoI
    aciI  maeII        csp6I    bslI   aciI      mseI hhaI/cfoI        maeIII  bbvI  maeIII                haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

        hinPI                                                                                      nlaIV
        hhaI/cfoI                                                mspI                             hgiJII
       haeII                                                     hpaII                            bsp1286
     rmaI    bsrBI                                              naeI                              bmyI
     maeI    aciI             mboII                    maeII  cfr10I              aluI            banII        nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                nlaIV                                             maeII     haeIII/palI
                hgiCI  taqI                                      draIII    sau96I                                    maeII
                banI mnlI                          hphI          bsaAI     asuI                                     drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

       pleI          tru9I     pleI                                  bslI
       hinfI   maeII  mseI      hinfI         bsrI                 bslI    avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                       thaI                                        hgiAI/aspHI
                                                       fnuDII/mvnI                                 bsp1286
                                                       bstUI                     maeII             bsiHKAI
                tru9I                                tru9I                     tru9I               bmyI    ddeI
                mseI               tru9I             mseI    apoI tru9I        mseI                apaLI/snoI  rsaI
         haeIII/palI       aluI     mseI      apoI   bsh1236I mseI      sspI  psp1406I             alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9M

```
                                                                                                              hinPI
                                                                                                              hhaI/cfoI
                                                                                                             thaI
                                                                                                             fnuDII/mvnI
                                                                                                             bstUI
                                                                           hinPI                                                       
                      aciI                                  maeIII       fnu4HI                                                       
                     fnu4HI     tru9I                      maeII bsrI  nlaIII  hhaI/cfoI               nspBII  bsh1236I
                  sfaNI         mseI        aciI          bsaAI tth111I/aspI bbvI            aciI      aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                                  thaI
                                                                                                                  fnuDII/mvnI
                                                                                                                  bstUI
                                                              scrFI                                               bsh1236I
                         mspI                                 nciI                                               hinPI
                         hpaII                                mspI                                               hhaI/cfoI
                        scrFI                            bsmAI         nspI                                     thaI
                        nciI     aciI                   esp3I hpaII fnu4HI                                      fnuDII/mvnI
                        dsaV sfaNI                     bslI   dsaV aluI nspHI                                   bstUI
       drdI             cauII fokI            aluI maeIII     cauII bbvI nlaIII    mnlI   hphI       hphI       bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                  mnlI
                                haeIII/palI                                                   maeII
                       mboII   sau96I                              nlaIII                    hinlI/acyI
                       bpuAI   asuI                          tru9I rcaI                      ahaII/bsaHI
       mnlI            bbsI   eco0109I/draII                 mseI  bspHI              ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                        nlaIV
                      aciI
                     thaI
                     fnuDII/mvnI
                     bstUI                                            rcaI
                     bsh1236I                                         bspHI
                    hinPI                                          bsrBI   bsmAI
                    hhaI/cfoI                                      aciI nlaIII                            sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

                   mboII                                          fnu4HI
                   earI/ksp632I                                   aciI                       hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9N

```
                                   hgiAI/aspHI
                                   bsp1286
                        sau3AI     bsiHKAI                      sau3AI  nspBII   sau3AI
                        mboI/ndeII(dam-)                        mboI/ndeII(dam-) mboI/ndeII(dam-)
                        dpnI(dam+) bmyI                         dpnI(dam+)       dpnI(dam+)
                        dpnII(dam-)                            bstYI/xhoII       dpnII(dam-)
                   eco57I          apaLI/snoI                bsrI dpnII(dam-)    alwI(dam-)
     hphI      sfaNI  mboII(dam-)  alw44I/snoI maeIII taqI   alwI(dam-)  aciI  bstYI/xhoII       mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                    scrFI
                                                                    nciI
                                               aciI                 mspI
                                               thaI                 hpaII
                                               fnuDII/mvnI          dsaV
      maeII             hgiAI/aspHI            bstUI                cauII
     psp1406I           bsp1286   tru9I        bsh1236I          hinlI/acyI
     xmnI               bsiHKAI   mseI        hinPI              hgaI                           aciI
     asp700             bmyI    ahaIII/draI   hhaI/cfoI          ahaII/bsaHI   bcgI   mcrI     fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                        rsaI
                        csp6I   bsrI                                                      fnu4HI
      ddeI             scaI hphI   maeIII     sfaNI    fokI    nlaIII                     bbvI       nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                          sau96I
                                          avaII                                              maeIII
                                   sau3AI asuI                                            nlaIII
             haeIII/palI           mboI/ndeII(dam-)                                     sau3AI
            eaeI                   dpnI(dam+)                                           mboI/ndeII(dam-)  sau3AI
            cfrI                   dpnII(dam-)                                          dpnI(dam+)        mboI/ndeII(dam-)
           fnu4HI                 pvuI/bspCI                                            dpnII(dam-)       dpnI(dam+)
           aciI                   mcrI    mnlI        aluI  aciI              nlaIII alwI(dam-)           dpnII(dam-)
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9O

```
                                                                                   hinPI
          mspI                                                                    mstI
           hpaII                                                                  aviII/fspI     bsrI
          bsaWI                                                       fnu4HI  maeII hhaI/cfoI  tru9I
     nlaIV     aluI                                 maeIII    sfaNI   bbvI      psp1406I        mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                      mspI
                       hpaII                                                                   bglI
                      scrFI                                                                   sau96I
                aluI  nciI        tru9I      fokI                        sau96I               haeIII/palI
              rmaI    dsaV        mseI   bsrI        aciI                avaII         hinPI  asuI    mspI
              maeI    cauII       aseI/asnI/vspI   mnlI                  asuI          hhaI/cfoI      hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                       mspI             thaI
                       hpaII            fnuDII/mvnI             haeIII/palI
                      cfr10I            bstUI                  sau96I
                  nlaIV  hphI        bsmAI aciI         fnu4HI  nlaIV
                gsuI/bpmI           bsaI bsh1236I      bbvI bsrI asuI          mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                                ddeI
                                           sau3AI       nlaIV
       pleI                                mboI/ndeII[dam-]  mnlI
       hinfI                               dpnI[dam+]   hgiCI        tru9I
    eam1105I              fokI             dpnII[dam-]  banI         mseI       maeIII
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                 rmaI     sau3AI
                                              sau3AI hphI mboI/ndeII[dam-]
                                              mboI/ndeII[dam-]
                                              dpnI[dam+]  dpnI[dam+]
                                     tru9I    dpnII[dam-) dpnII[dam-]
                   tru9I           ahaIII/draI maeI       alwI[dam-]       nlaIII             maeII
                   mseI        tru9I        bstYI/xhoII  bstYI/xhoII      rcaI             tru9I
                  ahaIII/draI  mseI mseI    alwI[dam-] mboII[dam-]        bspHI            mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9P

```
                                                sau3AI
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]  sau3AI          thaI
                                                dpnII[dam-] mboI/ndeII[dam-]
                                               bstYI/xhoII  dpnI[dam+]      fnuDII/mvnI
                                     sau3AI    alwI[dam-]   dpnII[dam-]     bstUI
                                     mboI/ndeII[dam-]       alwI[dam-]      bsh1236I
                 hgaI                dpnI[dam+] mboII[dam-]                hinPI       fnu4HI
             ddeI                    dpnII[dam-]           bstYI/xhoII     hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                              sau3AI
                              mboI/ndeII[dam-]
                              dpnI[dam+]
                              dpnII[dam-]
                             alwI[dam-]
             aciI            mspI                                     bsrI            hinPI
       aciI  nspBII          hpaII      aluI                      maeIII    eco57I    hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                     fnu4HI
                                                                                                alwNI  bbvI
       rmaI              haeIII/palI                                                          bsrI  fnu4HI
       maeI      bslI   haeI                 scfI     aciI        mnlI                 maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                 scrFI                                         aciI                      hgiAI/aspHI
                 nciI                                        nspBII                      bsp1286
                 mspI                         mspI          fnu4HI                       bsiHKAI
                 hpaII                        hpaII         bbvI                         bmyI
                 dsaV     pleI               bsaWI        hinPI mcrI                     apaLI/snoI
                 cauII    hinfI          maeIII           hhaI/cfoI                      alw44I/snoI  aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 9Q

```
                                                                                                       mspI
                                                              hinPI                                    hpaII
                                                              hhaI/cfoI                                bslI    fnu4HI
                           ddeI     scfI                     haeII                       aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                           scrFI
                                           mvaI          scrFI
                                           ecoRII  mvaI
                                           dsaV    ecoRII
                                           bstNI         dsaV
                        hinPI  mnlI        bsaJI         bstNI                                                taqI
                        hhaI/cfoI    aluI  apyI[dcm+]    apyI[dcm+]                            mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                    haeIII/palI
                                                                haeIII/palI     scrFI
                                                              fnu4HI            mvaI
                                                              aciI              ecoRII
                                                             thaI bslI          dsaV                   nlaIII
                                                             fnuDII/mvnI        bstNI bslI                  nspI
                                nlaIV                        bstUI              apyI[dcm+]       haeIII/palI  nspHI
            sfaNI             aciI                           bsh1236I       nlaIV haeI        haeI         aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                     fnu4HI              fnu4HI
                                                                                     bbvI                bbvI pleI
                           tfiI                                            bsrBI   aciI               hinPI   hinfI
                           hinfI                    aciI          aluI     aciI  fnu4HI       mcrI    hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9R

```
                                                            thaI
                                                            fnuDII/mvnI
                                                            bstUI
                                                            bsh1236I
                                                          hinPI
                                                          hhaI/cfoI
                                                         thaI
                                                         fnuDII/mvnI
                      sapI  hinPI                        bstUI              tru9I   aluI
                      mboII hhaI/cfoI                    bsh1236I haeIII/palI      pvuII
                      earI/ksp632I              mnlI    bslI     eaeI   tfiI aseI/asnI/vspI
            mnlI   aciI    haeII            aciI        aciI     cfrI   hinfI mseI  nspBII             bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                  scrFI
                                                                  mvaI
                                                                  ecoRII
                                                                  dsaV
                                1tru9I                      nlaIV bstNI
                    hinPI       mseI      maeIII            hgiCI apyI[dcm+]             mspI
        aciI        hhaI/cfoI  aseI/asnI/vspI mnlI          banI  bsaJI                  hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                               tru9I
                                                               mseI
                                                              aseI/asnI/vspI
           aciI                                             xmnI
         bsrBI                          aluI      nlaIII    asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT

>length: 6557
```

FIG. 10A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286                                                                                     thaI
       bsiHKAI                                                                                     fnuDII/mvnJ
       bmyI                       rmaI    tru9I                                                    bstUI
       banII                      maeI    mseI                                                     bsh1236I
     taqI                        speI    aseI/asnI/vspI                           bslI            aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                      scrFI
                      mvaI
                      ecoRII
                      dsaV
                   aciI
                 bglI bstNI                                maeII
                sau96I                                   hinlI/acyI
                haeIII/palI     aciI                     ahaII/bsaHI
                asuI  apyI[dcm+]              aciI     aatII          maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                      maeII
      hinlI/acyI                                                                                 hinlI/acyI
      ahaII/bsaHI                                     rsaI                        rsaI           ahaII/bsaHI
      aatII                              bglI         csp6I            ndeI       csp6I          aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                      nlaIII
         bglI  dsaV                                                                           styI
        sau96I bstNI            rsaI                                         maeII                  ncoI
        haeIII/palI             csp6I                              rsaI     snaBI                   dsaI hphI  aciI
        asuI   apyI[dcm+]   bsrI  nlaIII                           csp6I    bsaAI                   bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 10B

```
                                                                                  maeII
                                                                                  hinlI/acyI                 nlaIV
               rsaI                         pleI                                  ahaII/bsaHI                hgiCI
               csp6I                 aciI   hinfI                bsmAI            aatII                      banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                          aluI
                                                                                                         sstI
                                                                                                         sacI
                                                                                                         hgiJII
                                                                                                         hgiAI/aspHI
                                                                                                         ecl136II
                                                                                                         bsp1286
                                                                                                         bsiHKAI
                                                                            rsaI                         bmyI
                                 maeIII     aciI       hgaI        aciI     csp6I      mnlI              banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                              haeIII/palI
                                                                                             mcrI
                                                                                             eagI/xmaIII/eclXI
                                                                                             eaeI
                                                                                             cfrI
                                                                                            fnu4HI
                                                                                            aciI
                                                                                           thaI
                                  esp3I                                                    fnuDII/mvnI
                             scrFI                                         sau96I       sacII/sstII
                             mvaI bsmAI                                          avaII        nspBII
                             ecoRII                                        asuI         kspI  scrFI
                             dsaV                                         nlaIV         dsaI  nciI
                             bstNI  hinlI/acyI                               scrFI       bglI bslI   mspI
                             apyI[dcm+]                                      nciI   sau3AI mnlI bstUI
                        sau3AI gsuI/bpmI                                     mspI   mboI/ndeII[dam-] hpaII
                        mboI/ndeII[dam-]                             mboII   hpaII  dpnI[dam+] bsaJI dsaV
                        dpnI[dam+]  hgaI   fokI                      bpuAI   dsaV   dpnII[dam-] bsh1236I
                        dpnII[dam-] ahaII/bsaHI             mnlI     bbsI    cauII  alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
                tfiI                                                                                fnu4HI
            aciI                                                                                    aciI
           thaI hinfI                                                     bstXI                    thaI
           fnuDII/mvnI                                  aciI          sau96I    styI               fnuDII/mvnI tru9I
           bstUI                                   maeII rsaI      pleI scfI haeIII/palI           bstUI       mseI
           bsh1236I                            maeIII     csp6I   scfI hinfI   asuI    bsaJI       bsh1236I   aseI/asnI/vspI
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                      sau96I
                                                                                                      avaII
                                                                                                      asuI
                                                                                                   scrFI
                                                                                                   mvaI
                                                                                                   ecoRII
                                                                                                   dsaV
                                                                                                   bstNI
                                          maeIII                                                   apyI[dcm+]
                                         hphI   scfI         fokI                             bslI bsaJI
 801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
     GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                       hinlI/acyI
                                                       ahaII/bsaHI
                                                       aatII
                                                    thaI                        taqI[dam-]
                                                    fnuDII/mvnI               sau3AI              bsmAI
                  aluI  taqI                        bstUI                     mboI/ndeII[dam-]   esp3I
      mnlI       hindIII                           aciI maeII                 dpnI[dam+]     tth111I/aspI
      bsaJI   ddeI      claI/bsp106            hphI bsh1236I    taqI          dpnII[dam-] taqI  hgaI          sfaNI
 901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAGTTCG GACAGCGTCT CCGACCTGAT
     TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                              hinPI
                                                                                          fnu4HI
       aluI               tfiI                                                            bbvI
     fnu4HI               hinfI                 taqI                                     aluI hhaI/cfoI
     bbvI       mnlI  mboII                 aluI          mnlI               aciI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                  hinPI
                                  hhaI/cfoI    mspI
                                 thaI         hpaII
                                aciI          mroI
                             haeIII/palI      bspMII
                            mcrI fnuDII/mvnI  bspEI
    sau3AI                  eagI/xmaIII/eclXI bsaWI
    mboI/ndeII(dam-)        eaeI bstUI     tfiI
    dpnI(dam+)              cfrI bsh1236I  hinfI                  ecoRI
    dpnII(dam-)         sfaNI  fnu4HI  bslI  accIII               apoI                      sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                           mcrI
                                                                                           eagI/xmaIII/eclXI
                                                                                           eaeI
                                                                            styI          fnu4HI
       hgiAI/aspHI                                                   thaI    ncoI    sau3AI  aciI
       bsp1286                                                       fnuDII/mvnI     mboI/ndeII(dam-)
       bsiHKAI                                                fnu4HI bstUI   dsaI    dpnI(dam+)    sau3AI
       bmyI                                                   bbvI  mcrI    haeIII/palI dpnII(dam-)  mboI/ndeII(dam-)
       apaLI/snoI                                            scfI   mspI bsh1236I bsaJI sfaNI  fnu4HI  dpnI(dam+)
       alw44I/snoI    maeII                           nspBII pstI   hpaII    mnlI nlaIII  pvuI/bspCI haeIII/palI
     bslI draIII   maeIII       bspMI                 aciI   bsgI  cfr10I aciI haeI  fokI  mcrI bbvI cfrI dpnII(dam-)
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                  sau96I                                                  thaI     sau3AI
                                  avaII                                                   fnuDII/mvnI   nlaIII
                                  asuI                                                    bstUI    mboI/ndeII(dam-)
                           sau96I rsrII/cspI                                              bsh1236I dpnI(dam+)
                   aciI    haeIII/palI  aciI    tfiI                                     hinPI    dpnII(dam-)
      ddeI        bsrBI    asuI     cpoI        hinfI             nlaIII          ndeI hhaI/cfoI  alwI(dam-)
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                 hinPI                                                  draIII
                                                 hhaI/cfoI                                             nlaIV
                                                thaI                                  bsaJI            hgiCI
                                                fnuDII/mvnI                         haeIII/palI        banI
                              tth111I/aspI      bstUI                   sfaNI      sau96I             mspI
        bsrI               drdI          hgaI   bsh1236I     taqI    aluI         asuI mnlI    bslI  hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
                     aciI
                    thaI
                    fnuDII/mvnI
               hgiAI/aspHI
               bsp1286
               bsiHKAI                                         aciI
               bmyI  bstUI                                    fnu4HI
               apaLI/snoI                                    haeIII/palI
               alw44I/snoI                                  eaeI         aciI        gsuI/bpmI            tfiI bslI
           mnlI      bsh1236I   nlaIV                       cfrI       nspBII      bsrI      mnlI           hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                          fnu4HI
                                                                                                         thaI
                                                                                                         fnuDII/mvnI
                                                                                                         bstUI
                                                                                                         bsh1236I
                                                                                                        sacII/sstII
                                                                                                        nspBII
                                                                                                        kspI
                                                                                      mspI              dsaI
                                                                                      hpaII             bsaJI
                                                                                     mroI               aciI
                                                                                     bspMII            fnu4HI
                                                                                     bspEI           sau3AI aciI
                                            dsaI                                     bsaWI           mboI/ndeII[dam-]
                                         haeIII/palI                   rsaI     aciI    fokI         dpnI[dam+]
                                 mboII  mnlI bsaJI          fnu4HI    csp6I  bsrBI    sfaNI    aluI   dpnII[dam-]
            mnlI             mboII   gsuI/bpmI              bbvI    maeII    taqI    mnlI  accIII    alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

          scrFI
          nciI
          mspI
          hpaII                                                                       aluI
          dsaV                                                                       fnu4HI                       hgaI
          cauII                                                                      bbvI      hinPI     taqI     drdI
       nlaIV                 aciI                          aluI    nincII/hindII taqI  sfaNI     hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
                    nlaIV
                  mspI
                  hpaII  scrFI
                  bslI   nciI
                 mroI    mspI
                 bspMII  hpaII
                 bspEI[dam-]
                 bsaWI   dsaV
                 accIII[dam-]
               sau3AI    cauII
               mboI/ndeII[dam-]
               dpnI[dam+]
               dpnII[dam-]
               alwI[dam-]
```

```
haeIII/palI
mcrI
eagI/xmaIII/eclXI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
bstUI
bsh1236I     sau96I          rsaI
hinPI        avaII           csp6I
hhaI/cfoI    asuI            scaI
```

```
rsaI
csp6I          aciI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT
```

```
scrFI
nciI
mspI
hpaII
dsaV
xmaI/pspAI
smaI
scrFI
nciI
dsaV
cauII
bsaJI
avaI
bsaJI
sau3AI
mboI/ndeII[dam-]                       sau96I
dpnI[dam+]                     aciI    haeIII/palI
dpnII[dam-]                  fnu4HI    asuI
alwI[dam-]                   bglI  nlaIII
nlaIV cauII                  sfiI  styI
bstYI/xhoII                  eaeI  ncoI
bamHI bsaJI ecoRI            cfrI  dsaI
alwI[dam-]  apoI          taqI haeIII/palI
                          claI/bsp106 bsaJI
```

```
hinlI/acyI                              mcrI
hgaI             mnlI                   bslI
ahaII/bsaHI    bsaJI                  sfaNI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                     aluI
                   fnu4HI                                                                          rmaI
                   bbvI          maeIII             sfaNI   apoI                              bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                            sau3AI
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]
                                            dpnII[dam-]
                                           pvuI/bspCI
                                           mcrI
                                          taqI[dam-]  tru9I                  haeIII/palI
                                         claI/bsp106[dam-]                  haeI
                                        sau3AI        mseI       fnu4HI styI
                                        mboI/ndeII[dam-]         bbvI   ncoI
                                        dpnI[dam+] xmnI        hinPI    dsaI
                                        dpnII[dam-]  aseI/asnI/vspI     bsaJI
                               nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

                                                                        nlaIV
      rsaI                                                            scrFI                                 sfaNI
      csp6I                                                           mvaI                                      ppu10I
     nlaIV                                                            ecoRII                               nsiI/avaIII
     kpnI                                                             dsaV                                nlaIII
     hgiCI                                                            bstNI                                     sphI
     banI                       aluI                                  apyI[dcm+]                                nspI
     asp718     mnlI           pvuII                                 bsaJI                                      nspHI
     acc65I   ddeI  aciI       nspBII
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                              nlaIV
                        scrFI        scrFI
                        mvaI               mvaI                                 ppu10I
                        ecoRII       ecoRII                            nsiI/avaIII
                        dsaV         dsaV                             nlaIII
                        bstNI              bstNI                                sphI
                        apyI[dcm+]         apyI[dcm+]                           nspI sfaNI
                       sexAI              bsaJI                                 nspHI                         aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                    fnu4HI
                                                                                                    bglI
                                                                                                   sfiI
                                                                                                   haeIII/palI
                                                                     nlaIII                      mnlI
                                                                    styI                        haeIII/palI
                                                                    ncoI                      mnlI bsaJI aciI
                       aciI                bsrI                bslI dsaI
              aciI fokI              aciI        aciI          aciI bsaJI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                      scrFI
                                                                                      nciI
                                                                                      mspI
                                                                                      hpaII
                                                                                      dsaV
                                                          styI                      haeIII/palI
                                                          bsaJI                    mcrI
                                                          blnI                     eagI/xmaIII/eclXI
                                                       haeIII/palI        rmaI     eaeI
         haeIII/palI                                   stuI rmaI          maeI     cfrI
      mnlI       ddeI                   mnlI           haeI maeI         nheI      mspI cauII
      bsaJI mnlI   aluI              mnlI             mnlI avrII       aluI aluI   hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

         tfiI
         hinfI                                                                           fnu4HI
       aciI                                                                              aciI
      thaI                                                           bstXI              thaI
      fnuDII/mvnI                       aciI         scfI  sau96I   styI                fnuDII/mvnI  tru9I
      bstUI               pleI         rsaI        pleI   haeIII/palI                  bstUI        mseI
      bsh1236I            hinfI       csp6I  scfI  hinfI    asuI     bsaJI             bsh1236I  aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                  sau3AI
                  mboI/ndeII[dam-]
                  dpnI[dam+]                                                   sau96I
                  dpnII[dam-]                                                  avaII
                  alwI[dam-]                                                   asuI
                taqI[dam-]                                                  scrFI
              claI/bsp106[dam-]                                             mvaI                             rmaI
             sau3AI                                                         ecoRII             thaI    maeI
             mboI/ndeII[dam-]                                               dsaV               fnuDII/mvnI
             dpnI[dam+]                                                     bstNI                    bstUI  nheI
             dpnII[dam-]                                                    apyI[dcm+]       mnlI    bsh1236I  aluI
            alwI[dam-]              fokI                             bslI  bsaJI             bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                                nlaIII
                               styI
                               pflMI
                               ncoI
            sfaNI     ecoRI    dsaI                                                   rsaI              aluI
         fnu4HI taqI  apoI     bslI fokI                      rmaI             gsuI/bpmI             pvuII tth111I/aspI
         bbvI claI/bsp106      bsaJI       nlaIII  fokI       maeI            bsrI   csp6I     ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

            aluI
           sstI
           sacI
           hgiJII
           hgiAI/aspHI
           ecl136II
           bsp1286
           bsiHKAI
           bmyI                               hphI
           banII           mnlI              maeIII    bspMI                   taqI                          bsrI
        avaI          aciI                  bstEII  hphI        bsrI     hgaI             hphI    aluI  nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

```
                                                                                      mspI
                                                                                      hpaII
                                                                                      bslI
                                                  gsuI/bpmI                           bsaWI
                                                  scrFI                         sau3AI
                                                  mvaI                                mboI/ndeII[dam-]
                                           haeIII/palI                                dpnI[dam+]
                                       fnu4HI    ecoRII                    dpnII[dam-]
         scrFI                             aciI       dsaV                      alwI[dam-]
         mvaI                            thaI mnlI    bstNI                    nlaIV
         ecoRII                          fnuDII/mvnI  apyI[dcm+]  pleI         bstYI/xhoII
         dsaV                                bstUI    rsaI   pleI gsuI/bpmI         bamHI
         bstNI   aluI                        bsh1236I csp6I  hinfI  hinfI           alwI[dam-]
         apyI[dcm+]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
     ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT

                                                                           sau3AI                  styI
                                                                           mboI/ndeII[dam-]        bsaJI
                                                                           dpnI[dam+]            rsaI
                                                                           dpnII[dam-]           csp6I
                                                                           alwI[dam-]           nlaIV
                              fnu4HI  mboII                               nlaIV                 kpnI
                              bbvI    bpuAI                               bstYI/xhoII           hgiCI
                            scfI      bbsI                                bamHI rsaI            banI
                            pstI    mspI                                mnlI    csp6I           asp718
                            bsgI    hpaII                          maeIII  alwI[dam-]           acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
     GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG

       sau3AI
       mboI/ndeII[dam-]               mboII
       dpnI[dam+]           fnu4HI   bpuAI        aciI
       dpnII[dam-]          bbvI     bbsI    mboII                          mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
     GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
                                                                          bstNI
                          haeIII/palI                                     apyI[dcm+]
   xmnI                   haeI    rsaI                mnlI
   asp700                mnlI     csp6I               bslI      maeIII  bsaJI       maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
     GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT
```

FIG. 10K

```
                                                                                                                    sstI
                                                                                                                    sacI
                                                                                                                    hgiJII
                                                                                                                    hgiAI/aspHI
                                                                                                                    ecl136II
                                                                                                                    bsp1286
                                                                                                                    bsiHKAI
                                                                                                                    bmyI
                                                                                                               haeIII/palI
                                                   ddeI                                                       sau96I aluI
                                                  celII/espI                                                  asuI  banII
                           ddeI fnu4HI            bpu1102I                                      hphI         eco0109I/draII
                   scfI   mnlI  bbvI           hgaI                           accI             maeIII    alwNI  ddeI
3401 AGGACAGCAC CTACAGCCTC AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA GTCACCCATC AGGGCCTGAG
     TCCTGTCGTG GATGTCGGAG TCGTCGTGGG ACTGCGACTC GTTTCGTCTG ATGCTCTTTG TGTTTCAGAT GCGGACGCTT CAGTGGGTAG TCCCGGACTC

                                                                       sau96I
                                                                    nlaIII
                                                                aciI   haeIII/palI
                                                              fnu4HI   asuI
                                                              bglI styI
                                                     aluI     sfiI ncoI
                                                    hindIII  eaeI dsaI                       aluI
                                                   tru9I     cfrI bsaJI                    fnu4HI
               maeIII     aluI                     mseI  taqI haeIII/palI                  bbvI         maeIII
3501 CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAA GCTTCGATGG CCGCCATGGC CCAACTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
     GAGCGGGCAG TGTTTCTCGA AGTTGTCCCC TCTCACAATT CGAAGCTACC GGCGGTACCG GGTTGAACAA ATAACGTCGA ATATTACCAA TGTTTATTTC

                                                                                                                    sau3AI
                                                                                                                    mboI/ndeII[dam-]
                                                                                                                    dpnI[dam+]
                                                                                                                    dpnII[dam-]
                                                                                                                   pvuI/bspCI
                                                                                                                   mcrI
                                                                                                                  taqI[dam-]
                                                                                                                 claI/bsp106[dam-]
                                                                                                                sau3AI
                                                                                                                mboI/ndeII[dam-]
                                                                                                                dpnI[dam+]
                                                           rmaI                                                 dpnII[dam-]
                 sfaNI   apoI                         bsmI maeI                                     nlaIII    alwI[dam-]
3601 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCGAT
     GTTATCGTAG TGTTTAAAGT GTTTATTTCG TAAAAAAAGT GACGTAAGAT CAACACCAAA CAGGTTTGAG TAGTTACATA GAATAGTACA GACCTAGCTA
```

FIG. 10L

```
                                                                  rsaI
                              haeIII/palI                         csp6I
                             haeI                                nlaIV
          tru9I     fnu4HI styI                                  kpnI
          mseI      bbvI   ncoI                                  hgiCI
         aseI/asnI/vspI    dsaI                                  banI                    aluI
       xmnI       hinPI    bsaJI                                 asp718    mnlI         pvuII
       asp700     hhaI/cfoI nlaIII       mnlI     mnlI           acc65I ddeI aciI       nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                               nlaIV                                                                      nlaIV
                            scrFI                        sfaNI                scrFI       scrFI
                            mvaI                            ppu10I                 mvaI                mvaI
                            ecoRII                     nsiI/avaIII            ecoRII      ecoRII
                            dsaV                      nlaIII                  dsaV        dsaV
                            bstNI                        sphI                      bstNI               bstNI
                            apyI[dcm+]                   nspI                      apyI[dcm+]          apyI[dcm+]
                           bsaJI                         nspHI                    sexAI               bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                 ppu10I
                                 nsiI/avaIII
                                nlaIII
                               sphI
                               nspI sfaNI                                                          bsrI
                               nspHI                          aciI        aciI fokI aciI        aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                             fnu4HI
                                                             bglI
                       nlaIII                               sfiI
                      styI                                  haeIII/palI
                      ncoI                                mnlI    mnlI     ddeI
                 bslI dsaI                             haeIII/palI bsaJI mnlI aluI                mnlI
      aciI       aciI bsaJI                         mnlI bsaJI aciI    haeIII/palI               mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

```
                 styI                                                                          scrFI
                 bsaJI                                                                         mvaI
                 blnI                                                                          ecoRII
              haeIII/palI                tru9I            haeIII/palI                          dsaV
             stuI rmaI                   mseI            eaeI                                  bstNI
             haeI maeI                  hpaI  aluI       cfrI                   maeIII         apyI[dcm+]       tru9I
            mnlI avrII             aluI hincII/hindII  bsrI                maeII    bsrI       bsaJI  maeIII     mseI
4101 TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG CTGTTAACAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT
     AAAAAACCTC CGGATCCGAA AACGTTTTTC GACAATTGTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA

                                                                        sau3AI
                                                                        mboI/ndeII[dam-]
                                                             sau96I     dpnI[dam+]
                                                             haeIII/palI
                                         aluI             mnlI  aciI    dpnII[dam-]
                fnu4HI                pvuII            mboII asuI      pvuI/bspCI
                bbvI  fokI            nspBII           earI/ksp632I    mcrI                       bglI
4201 TAATCGCCTT GCAGCACATC CCCCCTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT TGCGTAGCCT GAATGGCGAA
     ATTAGCGGAA CGTCGTGTAG GGGGGAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCATCGGA CTTACCGCTT

       hinPI
       hhaI/cfoI
      nlaIV                                                                              hinPI
      narI                                                                               hhaI/cfoI
      kasI                                                                              thaI
      hinlI/acyI                                                                        fnuDII/mvnI              hinPI
      hgiCI                                                                             bstUI           hinPI    hhaI/cfoI
      haeII      aciI                                                                   bsh1236I        hhaI/cfoI
      banI   sfaNI                                                                   rsaI      scfI fnu4HI   tru9I
      ahaII/bsaHI                  sfaNI    aciI           aciI  maeII               csp6I   bslI   aciI     mseI
4301 TGGCGCCTGA TGCGGTATTT TCTCCTTACG CATCTGTGCG GTATTTCACA CCGCATACGT CAAAGCAACC ATAGTACGCG CCCTGTAGCG GCGCATTAAG
     ACCGCGGACT ACGCCATAAA AGAGGAATGC GTAGACACGC CATAAAGTGT GGCGTATGCA GTTTCGTTGG TATCATGCGC GGGACATCGC CGCGTAATTC

                              fnu4HI
         aciI               hinPI
       fnu4HI               hhaI/cfoI                            hinPI
      aciI                 thaI                                  hhaI/cfoI
     thaI                  fnuDII/mvnI                          haeII
     fnuDII/mvnI           bstUI                        hinPI rmaI
     bstUI                 bsh1236I     aciI            hhaI/cfoI    bsrBI
     bsh1236I         maeIII  bbvI maeIII              haeII  maeI   aciI             mboII                 maeII
4401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCTTTCT CGCCACGTTC
     GCGCCGCCCA CACCACCAAT GCGCGTCGCA CTGGCGATGT GAACGGTCGC GGGATCGCGG GCGAGGAAAG CGAAAGAAGG GAAGGAAAGA GCGGTGCAAG
```

FIG. 10N

```
                                 nlaIV
      mspI                       hgiJII                                        mnlI
      hpaII                      bsp1286                                     nlaIV
     naeI                        bmyI                                        hgiCI
     cfr10I         aluI         banII          nlaIV                        banI    taqI                 hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

        maeII    haeIII/palI
       draIII   sau96I                               maeII  pleI        tru9I     pleI
       bsaAI    asuI                                drdI    hinfI maeII  mseI      hinfI       bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                           thaI
                                                                                                           fnuDII/mvnI
                                                                                                           bstUI
                                                                  tru9I                                    bsh1236I
         bslI                                                     mseI                  tru9I        tru9I
        bslI    avaI                                        haeIII/palI        aluI     mseI    apoI mseI     apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                  hgiAI/aspHI
                                  bsp1286
                 maeII            bsiHKAI                                                                    tth111I/aspI
               tru9I              bmyI    ddeI                         aciI                                maeIII
      tru9I    mseI               apaLI/snoI  rsaI                    fnu4HI      tru9I                   maeII
      mseI   sspI  psp1406I       alw44I/snoI csp6I               sfaNI        mseI        aciI          bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                           hinPI
                                           hhaI/cfoI                    mspI
                                          thaI                          hpaII
               hinPI                      fnuDII/mvnI                  scrFI                                bsmAI
             fnu4HI                       bstUI                        nciI     aciI                       esp3I
             bbvI                   nspBII  bsh1236I                   dsaV sfaNI                            bslI
         nlaIII  hhaI/cfoI    aciI  aciI  hgaI        drdI             cauII fokI              aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                                    thaI
                                                                    fnuDII/mvnI
                                                                    bstUI
                                                                    bsh1236I
      scrFI                                                        hinPI
      nciI                                                         hhaI/cfoI                                  mnlI
      mspI      nspI                                              thaI                                      haeIII/palI
      hpaII fnu4HI                                                fnuDII/mvnI          mboII               sau96I
      dsaV aluI nspHI                                             bstUI mnlI           bpuAI               asuI
      cauII bbvI nlaIII     mnlI    hphI       hphI               bsh1236I             bbsI               eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                                  nlaIV
                                                                                 aciI
                                                                                thaI
                                                                                fnuDII/mvnI
                                         maeII                                  bstUI
              nlaIII                    hinlI/acyI                              bsh1236I
        tru9I rcaI                      ahaII/bsaHI                            hinPI
        mseI  bspHI                ddeI aatII                                  hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGGATAAACA AATAAAAAGA TTTATGTAAG

                  rcaI
                  bspHI
              bsrBI   bsmAI
              aciI nlaIII                                   sspI            mboII
                                                                            earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                                 hgiAI/aspHI
                                                                                                 bsp1286
                                                                                       sau3AI    bsiHKAI
                                                                                       mboI/ndeII[dam-]
                                                                                       dpnI[dam+] bmyI
                                                                                       dpnII[dam-]
             fnu4HI                                                               eco57I          apaLI/snoI
             aciI                       hphI             hphI                 sfaNI mboII[dam-]   alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
              sau3AI
              mboI/ndeII[dam-]    sau3AI
              dpnI[dam+]          mboI/ndeII[dam-]
              dpnII[dam-]         dpnI[dam+]                psp1406I          hgiAI/aspHI                    aciI
             bstYI/xhoII          dpnII[dam-]              xmnI              bsp1286   tru9I                 thaI
          bsrI        nspBII      alwI[dam-]               asp700            bsiHKAI   mseI                  fnuDII/mvnI
      taqI   alwI[dam-]  aciI    bstYI/xhoII           mboII maeII           bmyI    ahaIII/draI             bstUI
                                                                                                             bsh1236I
                                                                                                             hinPI
                                                                                                             hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                  scrFI
                  nciI
                  mspI
                  hpaII
                  dsaV
                  cauII
               hinlI/acyI                                                     rsaI
               hgaI                   aciI                                    csp6I  bsrI
               ahaII/bsaHI   bcgI   mcrI fnu4HI          ddeI                 scaI hphI   maeIII    sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                sau96I
                                                                                                avaII
                                                                                          sau3AI  asuI
                                                                  haeIII/palI             mboI/ndeII[dam-]
                                                                 eaeI                     dpnI[dam+]
                                                                 cfrI                     dpnII[dam-]
                                       fnu4HI                    fnu4HI                  pvuI/bspCI
      fokI  nlaIII                     bbvI        nlaIII        aciI                    mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                    nlaIII
                                 sau3AI maeIII                         mspI
                                 mboI/ndeII[dam-]    sau3AI   nlaIV
                                 dpnI[dam+]          mboI/ndeII[dam-]
                                alwI[dam-]           dpnI[dam+]    hpaII
     aluI   aciI              nlaIII dpnII[dam-]     dpnII[dam-] bsaWI aluI                               maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                               mspI
                              hinPI                                            hpaII
                            mstI                                               scrFI
                            aviII/fspI      bsrI                   aluI ncII          tru9I      fokI
           fnu4HI        maeII hhaI/cfoI  tru9I                   rmaI  dsaV          mseI    bsrI       aciI
      sfaNI  bbvI        psp1406I         mseI                    maeI  cauII        aseI/asnI/vspI   mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                          aciI
                             bglI                                         mspI           thaI
                           sau96I                                         hpaII          fnuDII/mvnI
             sau96I        haeIII/palI                                  cfr10I           bstUI
             avaII      hinPI asuI     mspI                           nlaIV  hphI      bsmAI          fnu4HI
             asuI       hhaI/cfoI      hpaII                        gsuI/bpmI          bsaI bsh1236I  bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                ddeI
          sau96I                                                                              sau3AI
          asuI                                       pleI                                     mboI/ndeII[dam-]
         nlaIV                                       hinfI                                    dpnI[dam+]
      bsrI haeIII/palI      mnlI                  eam1105I          fokI                      dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                      hphI
                                                                                                    rmaI
                                                                                                  sau3AI
                                                                                                  mboI/ndeII[dam-]
                                                                                                  dpnI[dam+]
       mnlI                                                                               tru9I   dpnII[dam-]
     nlaIV                                                                tru9I          ahaIII/draI maeI
     hgiCI        tru9I                                                   mseI         tru9I     bstYI/xhoII
     banI         mseI      maeIII                                       ahaIII/draI   mseI mseI  alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
                                                                                                    sau3AI
   sau3AI                                                                                           mboI/ndeII(dam-)
   mboI/ndeII(dam-)                                                                                 dpnI(dam+)
   dpnI(dam+)                                                                                       dpnII(dam-)
   dpnII(dam-)                                                                                     bstYI/xhoII
   alwI(dam-)                                                                              sau3AI  alwI(dam-)
  bstYI/xhoII          nlaIII          maeII                                               mboI/ndeII(dam-)
 mboII(dam-)          rcaI            tru9I                                                dpnI(dam+) mboII(dam-)
                      bspHI           mseI                    ddeI hgaI                    dpnII(dam-)
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                 thaI                                                                sau3AI
  sau3AI         fnuDII/mvnI                                                         mboI/ndeII(dam-)
  mboI/ndeII(dam-)                                                                   dpnI(dam+)
  dpnI(dam+)     bstUI                                                               dpnII(dam-)
  dpnII(dam-)    bsh1236I                                                           alwI(dam-)
  alwI(dam-)     hinPI        fnu4HI                               aciI             mspI
 bstYI/xhoII     hhaI/cfoI    bbvI                          aciI  nspBII            hpaII      aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

         bsrI          hinPI                  rmaI                haeIII/palI
      maeIII    eco57I     hhaI/cfoI          maeI       bslI     haeI                 scfI       aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                   scrFI
                                                                   nciI
                                    fnu4HI                         mspI                     mspI
                             alwNI  bbvI                           hpaII                    hpaII
                            bsrI  fnu4HI                           dsaV     pleI           bsaWI      hinPI
   mnlI                  maeIII   bbvI    bsrI                     cauII    hinfI        maeIII       hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                             hgiAI/aspHI
   mcrI                      bsp1286
   aciI                      bsiHKAI
  nspBII                     bmyI                                                                     hinPI
 fnu4HI                      apaLI/snoI                                                               hhaI/cfoI
 bbvI                        alw44I/snoI   aluI                       ddeI     scfI                  haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

```
                                                                                                      scrFI
                                                                                                      mvaI         scrFI
                                                                                                      ecoRII  mvaI
                                          mspI                                                        dsaV    ecoRII
                                          hpaII                                                       bstNI        dsaV
                                          bslI     fnu4HI                      hinPI   mnlI           bsaJI        bstNI
                               aciI       bsaWI    aciI                        hhaI/cfoI    aluI      apyI[dcm+]   apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                fnu4HI
                                                                                                                aciI
                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                   taqI                                    nlaIV               bstUI
                                      mnlI  drdI   hgaI            sfaNI                  aciI                 bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                    haeIII/palI
                 scrFI
                 mvaI
                 ecoRII
                 dsaV                  nlaIII
                 bstNI bslI                 nspI
      bslI       apyI[dcm+]    haeIII/palI  nspHI                       tfiI
    haeIII/palI nlaIV haeI   haeI           aflIII                      hinfI                   aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                                              thaI
                                                                                                                              fnoDII/mvnI
                                                                                                                              bstUI
                                                                                                                              bsh1236I
                                                                                                                             hinPI
                                                                                                                             hhaI/cfoI
                                                                                             hinPI                          thaI
                       fnu4HI             fnu4HI                                        sapI hhaI/cfoI                      fnuDII/mvnI
                       bbvI               bbvI  pleI                                    mboII                               bstUI
             bsrBI  aciI                hinPI   hinfI                                  earI/ksp632I                         bsh1236I
     aluI    aciI  fnu4HI        mcrI   hhaI/cfoI                   mnlI    aciI   haeII                     aciI   mnlI    bslI
                                                                                                                           aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                tru9I    aluI
      haeIII/palI        pvuII                                                         tru9I
     eaeI tfiI   mseI    nspBII                                        hinPI           mseI      maeIII            nlaIV
     cfrI hinfI aseI/asnI/vspI              bsrI      aciI            hhaI/cfoI  aseI/asnI/vspI mnlI               hgiCI
                                                                                                                    banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

      scrFI
      mvaI
      ecoRII
      dsaV
      bstNI
      apyI(dcm+)              mspI                          aciI                                                xmnI
     bsaJI                    hpaII                       bsrBI                                 aluI    nlaIII   asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors' see page 192, line 7 - page 196, line 5; figures 2,3 --- -/--	1-3,7,8 5,6, 9-12, 16-21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 37, no. 3, July 1984 CELL PRESS,CAMBRIDGE,MA,US;, pages 1053-1062, C.L. CEPKO ET AL. 'Construction and applications of a highly transmissible murine retrovirus shuttle vector' cited in the application	1-3,7,8
Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21
Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21
Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21
Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21
A	WO,A,92 17566 (GENENTECH INC) 15 October 1992 cited in the application see the whole document	1-21
A	WO,A,90 12025 (UNIV LELAND STANFORD JUNIOR) 18 October 1990 cited in the application see the whole document	1-21
A	EP,A,0 260 148 (GENENTECH INC) 16 March 1988 see the whole document	1-21
A	EP,A,0 160 457 (GENENTECH INC) 6 November 1985 cited in the application see the whole document	1-21

-/--

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document -----	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 95/09576

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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		DE-A-	3688900	23-09-93
		DE-T-	3688900	09-12-93
		JP-A-	62111690	22-05-87
		US-A-	4959318	25-09-90
		US-A-	5073609	17-12-91
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		JP-T-	6506356	21-07-94
WO-A-9012025	18-10-90	US-A-	5043270	27-08-91
EP-A-0260148	16-03-88	AU-B-	613316	01-08-91
		AU-B-	7831787	19-05-88
		DE-A-	3730599	07-07-88
		FR-A-	2603899	18-03-88
		GB-A,B	2197321	18-05-88
		US-A-	5024939	18-06-91
		JP-A-	63152986	25-06-88
EP-A-0160457	06-11-85	AU-B-	601358	13-09-90
		AU-B-	4134585	24-10-85
		AU-B-	5295890	30-08-90
		EP-A-	0385558	05-09-90
		HK-A-	8395	27-01-95
		JP-A-	60243023	03-12-85
		JP-A-	6040942	15-02-94
		NO-B-	174934	25-04-94
		SG-A-	3994	10-06-94
		US-A-	4965199	23-10-90